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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US98/22375 <b>(22) International Filing Date:</b> 23 October 1998 (23.10.98)  <b>(30) Priority Data:</b> 60/063,147                      24 October 1997 (24.10.97)                      US  <b>(71) Applicant:</b> UNIVERSITY OF UTAH RESEARCH FOUNDATION [US/US]; Suite 170, 421 Wakara Way, Salt Lake City, UT 84108 (US).  <b>(72) Inventors:</b> SINGH, Nanda, A.; Timberlakes #101, Heber City, UT 84032 (US). LEPPERT, Mark, F.; 1466 Westminster Avenue, Salt Lake City, UT 84105 (US). CHARLIER, Carole; 7, rue de Blindel, B-4141 Sprimont (BE).  <b>(74) Agents:</b> IHNEN, Jeffrey, L. et al.; Rothwell, Figg, Ernst & Kurz, Columbia Square, Suite 701 East, 555 13th Street N.W., Washington, DC 20004 (US).		<b>(81) Designated States:</b> CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> KCNQ2 AND KCNQ3-POTASSIUM CHANNEL GENES WHICH ARE MUTATED IN BENIGN FAMILIAL NEONATAL CONVULSIONS (BFNC) AND OTHER EPILEPSIES  <b>(57) Abstract</b> <p>Generalized idiopathic epilepsies (IGE) cause 40 % of all seizures and commonly have a genetic basis. One type of IGE is Benign Familial Neonatal Convulsions (BFNC), a dominantly inherited disorder of newborns. A submicroscopic deletion of chromosome 20q13.3 which cosegregates with seizures in BFNC family has been identified. Characterization of cDNAs spanning the deleted region identified a novel voltage-gated potassium channel, KCNQ2, which belongs to a new KCNQ1-like class of potassium channels. Nine other BFNC probands were shown to have <i>KCNQ2</i> mutations including three missense mutations, three frameshifts, two nonsense mutations, and one splice site mutation. A second gene, <i>KCNQ3</i>, was found in a separate BFNC family in which the mutation had been localized to chromosome 8. A missense mutation was found in this gene in perfect cosegregation with the BFNC phenotype in this latter family. This demonstrates that defects in potassium channels can cause epilepsy. Furthermore, some members of one of the BFNC families with a mutation in <i>KCNQ2</i> also exhibited rolandic epilepsy and one individual with juvenile myoclonic epilepsy has a mutation in an alternative exon of <i>KCNQ3</i>.</p>		

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TITLE OF THE INVENTION

KCNQ2 and KCNQ3 - POTASSIUM CHANNEL GENES WHICH ARE MUTATED IN BENIGN FAMILIAL NEONATAL CONVULSIONS (BFNC) AND OTHER EPILEPSIES

This application was made with Government support under Grant Nos. R01-NS32666  
5 funded by the National Institutes of Health, Bethesda, Maryland.

CROSS REFERENCE TO RELATED APPLICATIONS

This application is related to Serial No. 60/063,147, filed 24 October 1997, to which priority  
is claimed and which is incorporated herein by reference.

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BACKGROUND OF THE INVENTION

Epileptic disorders affect about 20 to 40 million people worldwide. Generalized idiopathic epilepsies (IGE) cause 40% of all epileptic disorders and commonly have a genetic basis (Plouin, 1994). Most of the IGEs that are inherited are complex, non-monogenic diseases. One type of IGE  
15 is Benign Familial Neonatal Convulsions (BFNC), a dominantly inherited disorder of newborns (Ronen et al., 1993; Hauser and Kurland, 1975). BFNC (OMIM 121200) is an autosomal dominantly inherited epilepsy of the newborn infant. This idiopathic, generalized epilepsy typically has an onset of seizures on day two to four of life. Spontaneous remission of the seizures occurs between two to fifteen weeks (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975).  
20 Seizures typically start with a tonic posture, ocular symptoms and other autonomic features which then often progress to clonic movements and motor automatisms. These neonates thrive normally between the seizures, and their neurologic examinations and later development indicate normal brain functioning (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975). However, in spite of normal neurologic development, seizures recur later in life in approximately 16% of BFNC cases  
25 compared with a 2% cumulative lifetime risk of epilepsy in the general population (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975).

Genetic heterogeneity of BFNC has been observed (Ryan et al., 1991). Two loci, *EBN1* and *EBN2*, have been mapped by linkage analysis to chromosome 20q13 (Leppert et al., 1989; Malafosse et al., 1992) and chromosome 8q24 (Lewis et al., 1993; Steinlein et al., 1995),  
30 respectively.

The nomenclature of the genes of this invention as well as related genes has changed over time. Two of the genes of this invention from humans are now referred to as *KCNQ2* and *KCNQ3*.

These had originally been named *KVEBN1* and *KVEBN2*, respectively. The two sets of names are equivalent and can be used interchangeably, but the accepted nomenclature is now *KCNQ2* and *KCNQ3* and these names will be used herein. Also, the related gene *KCNQ1* had originally been called *KVLQT1* in the literature, but again the accepted name now is *KCNQ1* and this name will be used herein.

Linkage analysis in a large kindred demonstrated that a gene, herein called *KCNQ2*, responsible for BFNC maps to chromosome 20q13.3 close to the markers D20S20 and D20S19 (Leppert et al., 1989). Following the initial report, two centers confirmed linkage of BFNC to the same two genetic markers on chromosome 20, termed the EBN1 (epilepsy benign neonatal type 1) locus (Ryan et al., 1991; Malafosse et al., 1992; Steinlein et al., 1992). A more distal marker, D20S24, shows complete co-segregation with the BFNC phenotype in chromosome 20 linked families. Finding a distal flanking marker for the BFNC locus has not been successful probably because of its proximity to the telomere. This telomeric region is characterized by a high recombination rate between markers when compared to the physical distance (Steinlein et al., 1992). In fact, Steinlein et al. have demonstrated that the three markers D20S19, D20S20 and D20S24 are contained on the same 450 Mb Mlu I restriction fragment (Steinlein et al., 1992). All of the families in the present study used to find and study *KCNQ2* show linkage to chromosome 20q markers with LOD scores of greater than 3.0 or have probands with clinical manifestations consistent with BFNC (Leppert et al., 1993). Each subject and control signed a Consent for Participation in these studies approved by the Institutional Review Board for Human Subject Research at their home institution. To find a gene responsible for BFNC, we narrowed a BFNC region with a sub-microscopic deletion in a single family, identified candidate cDNAs in this deletion, and then searched for mutations in other BFNC families. The gene has been identified and sequenced. Several distinct mutations have been found in this gene. These include a large deletion, three missense mutations, three frameshift mutations, two nonsense mutations and one splice site mutation. One of these mutations is associated with rolandic epilepsy as described in the Examples below.

A second chromosomal locus, EBN2, has also been identified for BFNC. Lewis et al. (1993) demonstrated linkage to markers on chromosome 8q24 in a single Hispanic family affected with BFNC. Evidence for this second locus was also reported in a Caucasian pedigree (Steinlein et al., 1995). The gene, herein called *KCNQ3*, responsible for EBN2 was mapped to chromosome 8, between markers D8S256 and D8S284 on a radiation hybrid map (Lewis et al., 1995). *KCNQ3* has been identified as set out in the examples of the instant disclosure. *KCNQ3* was screened for

mutations in the large BFNC family previously linked to chromosome 8q24 in the same marker interval (Ryan et al., 1991; Lewis et al., 1993). A missense mutation was found in the critical pore region in perfect cosegregation with the BFNC phenotype. The same conserved amino acid is also mutated in *KCNQ1* in an LQT patient (Wang et al., 1996). Furthermore, the segment of mouse chromosome 15 that harbors the *stargazer* (*stg*) locus (Noebels et al., 1990; Letts et al., 1997) is homologous to the human 8q24 region and the *stg* phenotype is close to a common form of IGE, the absence epilepsy. *KCNQ2*, *KCNQ3* and other undiscovered genes of the same family of K<sup>+</sup> channels are strong candidates for other, more common IGEs. One individual with juvenile myoclonic epilepsy has been found who has a mutation in an alternative exon of *KCNQ3* as shown in the Examples below.

IGEs include many different types of seizures. Common IGEs include generalized tonic-clonic seizure (GTCS), absence epilepsy of childhood (AEC), juvenile absence epilepsy (JAE) and juvenile myoclonic epilepsy (JME). Reutens and Berkovic (1995) have shown that the boundaries between the different IGE syndromes are indistinct and suggest that neurobiological and possibly genetic relationships exist between these syndromes. Interestingly, using non-parametric linkage methods, Zara et al. (1995) obtained evidence for involvement of an epilepsy locus at chromosome 8q24 in a panel of families with multiple cases of IGEs. Furthermore, in a population study, Steinlein et al. (1997) recently described a weak allelic association at the *CHRNA4* locus, on chromosome 20q13.3, physically close to *KCNQ2*, in a group of unrelated patients with multiple forms of IGEs. Finally, an epileptic mutant mouse *stargazer* (*stg*) (Noebels et al., 1990) is a genetic model of spike wave epilepsy. This is a recessive mutation and the phenotype is related to a common form of human IGE, the absence epilepsy. *Stg* has been mapped on mouse chromosome 15 in a region homologous to the human 8q24 region. Screening the mouse homolog of *KCNQ3* for mutations in an affected mouse will assess the hypothesis that the same gene is responsible for both BFNC and *Stargazer* phenotypes.

The present invention is directed to both *KCNQ2* and *KCNQ3* and their gene products, mutations in the genes, the mutated genes, probes for the wild-type and mutated genes, and to a process for the diagnosis and prevention of BFNC. Each of the genes encodes a potassium channel protein. The instant work shows that some families with BFNC have mutations in either *KCNQ2* or *KCNQ3*. BFNC is diagnosed in accordance with the present invention by analyzing the DNA sequence of the *KCNQ2* and/or *KCNQ3* gene of an individual to be tested and comparing the respective DNA sequence to the known DNA sequence of a normal *KCNQ2* and/or *KCNQ3* gene.

Alternatively, the *KCNQ2* gene and/or *KCNQ3* gene of an individual to be tested can be screened for mutations which cause BFNC. Prediction of BFNC will enable practitioners to prevent this disorder using existing medical therapy. Furthermore, a mutation in *KCNQ2* has been found which is associated with rolandic epilepsy and a mutation in *KCNQ3* has been found which is associated with JME. These two forms of epilepsy may also be diagnosed in accord with the invention.

Mouse genes homologous to the human *KCNQ2* and *KCNQ3* have also been found and sequenced and the sequences are disclosed. The mouse *KCNQ2* gene has been only partially isolated and sequenced (shown as SEQ ID NO:88), the 3' end not yet having been found. The complete mouse *KCNQ3* gene has been isolated and sequenced (shown as SEQ ID NO:90).

The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference, and for convenience are respectively grouped in the appended List of References.

#### SUMMARY OF THE INVENTION

The present invention demonstrates a molecular basis of Benign Familial Neonatal Convulsions (BFNC) as well as for rolandic epilepsy and juvenile myoclonic epilepsy. More specifically, the present invention has determined that molecular variants of either the *KCNQ2* gene or *KCNQ3* gene cause or are involved in the pathogenesis of these three forms of epilepsy. Genotypic analyses show that *KCNQ2* is linked to BFNC in ten unrelated families and *KCNQ3* is linked to BFNC in one other family. Furthermore, one mutation in the *KCNQ2* gene in two individuals of one family has been associated with rolandic epilepsy and one individual with a mutation in *KCNQ3* has been diagnosed with juvenile myoclonic epilepsy. Analysis of the *KCNQ2* and *KCNQ3* genes will provide an early diagnosis of subjects with BFNC, rolandic epilepsy or JME. The diagnostic method comprises analyzing the DNA sequence of the *KCNQ2* and/or the *KCNQ3* gene of an individual to be tested and comparing it with the DNA sequence of the native, non-variant gene. In a second embodiment, the *KCNQ2* and/or *KCNQ3* gene of an individual to be tested is screened for mutations which cause BFNC, rolandic epilepsy or JME. The ability to predict these epilepsies will enable physicians to prevent the disease with medical therapy such as drugs which directly or indirectly modulate K<sup>+</sup> ion channels.

The invention shows that various genetic defects of a potassium channel are responsible for the human idiopathic epilepsy of BFNC, rolandic epilepsy and/or JME. This finding adds to the growing list of channelopathies in humans (Ptacek, 1997). Importantly, this result suggests that

drugs which directly or indirectly modulate K<sup>+</sup> ion channels will be helpful in the treatment of seizure disorders.

### BRIEF DESCRIPTION OF THE FIGURES

5 The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1. Southern blot of kindred 1547 (showing 4 generations listed as I, II, III and IV) genomic DNA cut with *TaqI* and probed with the VNTR marker D20S24 showing a null allele in 10 affected individuals. Line A shows genotype misinheritances shown in boxes; line B shows corrected genotypes. The "N" indicates non-penetrant individuals.

Figures 2A-C. Metaphase spreads of cell lines from affected individuals of kindred 1547 probed with P1-KO9-7 (Figure 2C) and P1-KO9-6b (Figure 2B) genomic P1 clones and the 12 kb D20S24 RFLP marker (Figure 2A) demonstrating a deletion of D20S24.

15 Figure 3. Amino acid alignment between human members (KCNQ2, KCNQ3 and KCNQ1) and the *C. elegans* homologue (nKQT1) of the KQT-like family. The six transmembrane domains and the pore are indicated by a solid line located above the corresponding sequence. The conserved charged amino acids in the transmembrane domains are highlighted in gray. The sequence of KCNQ2 is SEQ ID NO:2, the sequence of KCNQ3 is SEQ ID NO:7, the sequence of nKQT1 is SEQ 20 ID NO:3 and the sequence of KCNQ1 is SEQ ID NO:4.

Figure 4. Figure 4 shows a three generation pedigree with BFNC linked to chromosome 20. BFNC individuals are depicted by filled in black circles and squares. The data is from kindred 1504 which shows variants in the KCNQ2 pores. The lower portion of the figure shows the cosegregation of the variant form which is present only in affected individuals. Sequence analysis revealed the 25 existence of a two base pair insertion in affected individuals showing the upper two (variant) bands.

Figure 5. Radiation Hybrid Mapping of the *KCNQ3* locus. Interpair LOD scores are given above the center line and distance between marker pairs, in cR<sub>5000</sub>, is shown below. The odds against inversion for adjacent loci is also given for each marker pair.

Figure 6. Figure 6 shows a three generation pedigree with BFNC linked to chromosome 8. 30 BFNC individuals are depicted by filled in black circles and squares. The non-penetrant individual III-8 is indicated by the symbol NP. The lower portion of the figure shows the co-segregation of the 187 bp SSCP variant, present only in affected and non-penetrant individuals (arrow).

Figures 7A-O. Intron/exon sequence is shown for *KCNQ2*. Exon sequence is shown in bold and primer sequence is in italics. The primer sequences are found in Table 4. The sequences are SEQ ID NOs:100-114.

Figures 8A-O. Intron/exon sequence is shown for *KCNQ3*. Exon sequence is shown uppercase and intron is shown lowercase and primer sequences are underlined. The primer sequences are found in Table 5. The sequences are SEQ ID NOs:115-129. Figure 8I shows the alternatively spliced exon found in a JME patient. Figure 8N shows an "N" in the 3' intron region. This "N" stands for Alu repeats which are found in this region.

#### 10 BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 is the cDNA sequence for *KCNQ2*.

SEQ ID NO:2 is the amino acid sequence for *KCNQ2*.

SEQ ID NO:3 is the amino acid sequence for nKQT1.

SEQ ID NO:4 is the amino acid sequence for *KCNQ1*.

15 SEQ ID NO:5 is nucleotide sequence at the intron/exon junction of the 3' end of the intron interrupting the two exons which encode amino acid 544 of *KCNQ2*.

SEQ ID NO:6 is the cDNA sequence for *KCNQ3*.

SEQ ID NO:7 is the amino acid sequence for *KCNQ3*.

SEQ ID NOs:8-9 are primers used for somatic cell hybrid panel genotyping (Example 7).

20 SEQ ID NOs:10-11 are primers used for genotyping a chromosome 8 radiation hybrid panel (Example 8).

SEQ ID NOs:12-17 are primers used to perform RACE to obtain full length cDNA (Example 9).

SEQ ID NOs:18-19 are primers used to prepare a PCR fragment which identified an SSCP variant for *KCNQ3*.

25 SEQ ID NOs:20-21 are hypothetical nucleic acid sequences to demonstrate calculation of percent homology between two nucleic acids.

SEQ ID NOs:22-53 are primers for amplifying portions of *KCNQ2*.

SEQ ID NOs:54-87 are primers for amplifying portions of *KCNQ3*.

SEQ ID NO:88 is a partial mouse *KCNQ2*.

30 SEQ ID NO:89 is a partial mouse *KCNQ2* encoded by SEQ ID NO:88.

SEQ ID NO:90 is a mouse *KCNQ3*.

SEQ ID NO:91 is the mouse *KCNQ3* encoded by SEQ ID NO:90.



SEQ ID NO:92 is an alternative exon found in *KCNQ3*.

SEQ ID NOs:93-94 are primers based on mouse sequence to amplify 5' end of human *KCNQ3*.

SEQ ID NO:95 is a mutated human *KCNQ2* with a GGGCC insertion after nucleotide 2736.

SEQ ID NO:96 is a mutated human *KCNQ2* encoded by SEQ ID NO:95.

5 SEQ ID NOs:97-99 are primers for amplifying portions of *KCNQ2*.

SEQ ID NOs:100-114 are intron/exon sequence for *KCNQ2* (Figures 7A-O).

SEQ ID NOs:115-129 are intron/exon sequence for *KCNQ3* (Figures 8A-O).

### DETAILED DESCRIPTION OF THE INVENTION

10 The present invention is directed to the determination that BFNC maps to the *KCNQ2* gene and to the *KCNQ3* gene and that molecular variants of these genes cause or are involved in the pathogenesis of BFNC, rolandic epilepsy and/or JME. More specifically, the present invention relates to mutations in the *KCNQ2* gene and in the *KCNQ3* gene and their use in the diagnosis of BFNC, rolandic epilepsy and JME. The present invention is further directed to methods of  
15 screening humans for the presence of *KCNQ2* and/or *KCNQ3* gene variants which cause BFNC, rolandic epilepsy and/or JME. Since these forms of epilepsy can now be detected earlier (i.e., before symptoms appear) and more definitively, better treatment options will be available in those individuals identified as having BFNC, rolandic epilepsy or JME. The present invention is also directed to methods for screening for drugs useful in treating or preventing BFNC, rolandic epilepsy  
20 or JME.

The present invention provides methods of screening the *KCNQ2* and/or *KCNQ3* gene to identify mutations. Such methods may further comprise the step of amplifying a portion of the *KCNQ2* or *KCNQ3* gene, and may further include a step of providing a set of polynucleotides which are primers for amplification of said portion of the *KCNQ2* or *KCNQ3* gene. The method is useful  
25 for identifying mutations for use in either diagnosis of or prognosis of BFNC, rolandic epilepsy and JME.

Benign Familial Neonatal Convulsion is an autosomal dominantly inherited disorder that causes epilepsy of the newborn infant. This idiopathic, generalized epilepsy typically has an onset of seizures on day two to four of life. Spontaneous remission of the seizures occurs between two  
30 to fifteen weeks (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975). Seizures typically start with a tonic posture, ocular symptoms and other autonomic features which then often progress to clonic movements and motor automatisms. These neonates thrive normally between the seizures,

and their neurologic examinations and later development indicate normal brain functioning (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975). However, in spite of normal neurologic development, seizures recur later in life in approximately 16% of BFNC cases compared with a 2% cumulative lifetime risk of epilepsy in the general population (Ronen et al., 1993; Plouin, 1994; 5 Hauser and Kurland, 1975).

Linkage analysis in a large kindred demonstrated that a gene responsible for BFNC maps to chromosome 20q13.3 close to the markers D20S20 and D20S19 (Leppert et al, 1989). Following the initial report, two centers confirmed linkage of BFNC to the same two genetic markers on chromosome 20, termed the EBN1 (epilepsy benign neonatal type 1) locus (Ryan et al., 1991; 10 Malafosse et al., 1992). A more distal marker, D20S24, shows complete co-segregation with the BFNC phenotype in chromosome 20 linked families. Finding a distal flanking marker for the BFNC locus has not been successful probably because of its proximity to the telomere. This telomeric region is characterized by a high recombination rate between markers when compared to the physical distance (Steinlein et al., 1992). In fact, Steinlein et al. have demonstrated that the three 15 markers D20S19, D20S20 and D20S24 are contained on the same 450 Mb Mlu I restriction fragment (Steinlein et al., 1992). All of the families in the present study for *KCNQ2* show linkage to chromosome 20q markers with LOD scores of greater than 3.0 or have probands with clinical manifestations consistent with BFNC (Leppert et al., 1993). To find this gene responsible for BFNC, we narrowed the BFNC region with a sub-microscopic deletion in a single family, identified 20 candidate cDNAs in this deletion, and then searched for mutations in other BFNC families.

A second chromosomal locus, EBN2, has also been identified for BFNC. Lewis et al. (1993) demonstrated linkage to markers on chromosome 8q24 in a single Hispanic family affected with BFNC. Evidence for this second locus was also reported in a Caucasian pedigree (Steinlein et al., 1995). The gene for EBN2, *KCNQ3*, has now been found and characterized as detailed in this 25 disclosure.

Finally, the present invention is directed to a method for screening drug candidates to identify drugs useful for treating or preventing BFNC, rolandic epilepsy or JME. Drug screening is performed by expressing mutant *KCNQ2* or mutant *KCNQ3* in cells, such as oocytes, mammalian cells or transgenic animals, and assaying the effect of a drug candidate on the *KCNQ2* or *KCNQ3* 30 potassium channel. The effect is compared to the *KCNQ2* or *KCNQ3* potassium channel activity obtained using the wild-type *KCNQ2* or *KCNQ3* gene.

Proof that the *KCNQ2* and *KCNQ3* genes are involved in causing BFNC, rolandic epilepsy and JME is obtained by finding sequences in DNA extracted from affected kindred members which create abnormal *KCNQ2* or abnormal *KCNQ3* gene products or abnormal levels of the gene products. Such susceptibility alleles will co-segregate with the disease in large kindreds. They will  
5 also be present at a much higher frequency in non-kindred individuals with epilepsy than in individuals in the general population. The key is to find mutations which are serious enough to cause obvious disruption to the normal function of the gene product. These mutations can take a number of forms. The most severe forms would be frame shift mutations or large deletions which would cause the gene to code for an abnormal protein or one which would significantly alter protein  
10 expression. Less severe disruptive mutations would include small in-frame deletions and nonconservative base pair substitutions which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic to an acidic amino acid or vice versa, from a hydrophobic to hydrophilic amino acid or vice versa, or other mutations which would affect secondary or tertiary protein structure. Silent mutations or those resulting in conservative  
15 amino acid substitutions would not generally be expected to disrupt protein function.

According to the diagnostic and prognostic method of the present invention, alteration of the wild-type *KCNQ2* or *KCNQ3* gene is detected. In addition, the method can be performed by detecting the wild-type *KCNQ2* or *KCNQ3* gene and confirming the lack of a cause of epilepsy as a result of this locus. "Alteration of a wild-type gene" encompasses all forms of mutations including  
20 deletions, insertions and point mutations in the coding and noncoding regions. Deletions may be of the entire gene or of only a portion of the gene. Point mutations may result in stop codons, frameshift mutations or amino acid substitutions. Somatic mutations are those which occur only in certain tissues and are not inherited in the germline. Germline mutations can be found in any of a body's tissues and are inherited. Point mutational events may occur in regulatory regions, such  
25 as in the promoter of the gene, leading to loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the *KCNQ2* or *KCNQ3* gene product, or to a decrease in mRNA stability or translation efficiency.

Useful diagnostic techniques include, but are not limited to fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded  
30 conformation analysis (SSCA), RNase protection assay, allele-specific oligonucleotide (ASO), dot blot analysis, hybridization using nucleic acid modified with gold nanoparticles and PCR-SSCP.

as discussed in detail further below. Also useful is the recently developed technique of DNA microchip technology.

The presence of BFNC, rolandic epilepsy or JME may be ascertained by testing any tissue of a human for mutations of the *KCNQ2* or *KCNQ3* gene. For example, a person who has inherited a germline *KCNQ2* mutation would be prone to develop BFNC. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the *KCNQ2* or *KCNQ3* gene. Alteration of a wild-type *KCNQ2* or *KCNQ3* allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing can detect sequence variation. Another approach is the single-stranded conformation polymorphism assay (SSCP) (Orita et al., 1989). This method does not detect all sequence changes, especially if the DNA fragment size is greater than 200 bp, but can be optimized to detect most DNA sequence variation. The reduced detection sensitivity is a disadvantage, but the increased throughput possible with SSCP makes it an attractive, viable alternative to direct sequencing for mutation detection on a research basis. The fragments which have shifted mobility on SSCP gels are then sequenced to determine the exact nature of the DNA sequence variation. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield et al., 1991), heteroduplex analysis (HA) (White et al., 1992) and chemical mismatch cleavage (CMC) (Grompe et al., 1989). None of the methods described above will detect large deletions, duplications or insertions, nor will they detect a regulatory mutation which affects transcription or translation of the protein. Other methods which might detect these classes of mutations such as a protein truncation assay or the asymmetric assay, detect only specific types of mutations and would not detect missense mutations. A review of currently available methods of detecting DNA sequence variation can be found in a recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific oligonucleotide (ASO) hybridization can be utilized to rapidly screen large numbers of other samples for that same mutation. Such a technique can utilize probes which are labeled with gold nanoparticles to yield a visual color result (Elghanian et al., 1997).

A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably with a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of BFNC, rolandic epilepsy or JME cases. Southern blots displaying hybridizing fragments differing in length from control DNA when probed with sequences near or including the *KCNQ2* locus indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis (PFGE) is employed.

Detection of point mutations may be accomplished by molecular cloning of the *KCNQ2* or *KCNQ3* allele and sequencing the allele using techniques well known in the art. Also, the gene or portions of the gene may be amplified, e.g., by PCR or other amplification technique, and the amplified gene or amplified portions of the gene may be sequenced.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis (SSCP) (Orita et al., 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell et al., 1990; Sheffield et al., 1989); 3) RNase protection assays (Finkelstein et al., 1990; Kinszler et al., 1991); 4) allele-specific oligonucleotides (ASOs) (Conner et al., 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and 6) allele-specific PCR (Ruano and Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular *KCNQ2* or *KCNQ3* mutation. If the particular mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

In the first three methods (SSCP, DGGE and RNase protection assay), a new electrophoretic band appears. SSCP detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a

denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

5 Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of samples. An example of a mismatch  
10 cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type *KCNQ2* or *KCNQ3* gene coding sequence. The riboprobe and either mRNA or DNA isolated from the person are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected  
15 by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the mRNA or gene, it will be  
20 desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., 1988; Shenk et al., 1975; Novack et al., 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA  
25 probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the *KCNQ2* or *KCNQ3* gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the *KCNQ2* or *KCNQ3* gene which have been amplified by use of PCR  
30 may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the gene sequence.

By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the gene. Hybridization of allele-specific probes with amplified *KCNQ2* or *KCNQ3* sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under high stringency hybridization conditions indicates the presence of the same mutation in the tissue as in the allele-specific probe. High stringency hybridization conditions are defined as those conditions which allow an 8 basepair stretch of a first nucleic acid (a probe) to bind to a 100% perfectly complementary 8 basepair stretch of nucleic acid while simultaneously preventing binding of said first nucleic acid to a nucleic acid which is not 100% complementary, i.e., binding will not occur if there is a mismatch.

The newly developed technique of nucleic acid analysis via microchip technology is also applicable to the present invention. In this technique, literally thousands of distinct oligonucleotide probes are built up in an array on a silicon chip. Nucleic acid to be analyzed is fluorescently labeled and hybridized to the probes on the chip. It is also possible to study nucleic acid-protein interactions using these nucleic acid microchips. Using this technique one can determine the presence of mutations or even sequence the nucleic acid being analyzed or one can measure expression levels of a gene of interest. The method is one of parallel processing of many, even thousands, of probes at once and can tremendously increase the rate of analysis. Several papers have been published which use this technique. Some of these are Hacia et al., 1996; Shoemaker et al., 1996; Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1996; Lipshutz et al., 1995. This method has already been used to screen people for mutations in the breast cancer gene BRCA1 (Hacia et al., 1996). This new technology has been reviewed in a news article in Chemical and Engineering News (Borman, 1996) and been the subject of an editorial (Nature Genetics, 1996). Also see Fodor (1997).

The most definitive test for mutations in a candidate locus is to directly compare genomic *KCNQ2* or *KCNQ3* sequences from patients with those from a control population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

Mutations from patients falling outside the coding region of *KCNQ2* or *KCNQ3* can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the genes. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in patients as compared to control individuals.

Alteration of *KCNQ2* or *KCNQ3* mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type gene. Alteration of wild-type genes can also be detected by screening for alteration of wild-type *KCNQ2* or *KCNQ3* protein. For example, monoclonal antibodies immunoreactive with *KCNQ2* or *KCNQ3* can be used to screen a tissue. Lack of cognate antigen would indicate a mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered *KCNQ2* or *KCNQ3* protein can be used to detect alteration of the wild-type *KCNQ2* or *KCNQ3* gene. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect *KCNQ2* or *KCNQ3* biochemical function. Finding a mutant *KCNQ2* or *KCNQ3* gene product indicates alteration of a wild-type *KCNQ2* or *KCNQ3* gene.

A mutant *KCNQ2* or *KCNQ3* gene or gene product can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant genes or gene products in tissues can be applied to other body samples. By screening such body samples, a simple early diagnosis can be achieved for BFNC, rolandic epilepsy or JME.

The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular *KCNQ2* or *KCNQ3* allele using PCR. The pairs of single-stranded DNA primers for *KCNQ2* or *KCNQ3* can be annealed to sequences within or surrounding the *KCNQ2* gene on chromosome 20 or *KCNQ3* gene on chromosome 8 in order to prime amplifying DNA synthesis of the gene itself. A complete set of these primers allows synthesis of all of the nucleotides of the gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele-specific primers can also be used. Such primers anneal only to particular *KCNQ2* or *KCNQ3* mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appended to their 5' ends. Thus, all nucleotides of the primers are derived from *KCNQ2* or *KCNQ3* sequence or sequences adjacent to *KCNQ2* or *KCNQ3*, except for the few nucleotides necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the



art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. Given the sequence of *KCNQ2* and *KCNQ3*, design of particular primers is well within the skill of the art.

The nucleic acid probes provided by the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase protection method for detecting point mutations already discussed above. The probes can be used to detect PCR amplification products. They may also be used to detect mismatches with the *KCNQ2* or *KCNQ3* gene or mRNA using other techniques.

It has been discovered that most individuals with the wild-type *KCNQ2* and *KCNQ3* genes do not have BFNC. However, mutations which interfere with the function of the *KCNQ2* or *KCNQ3* gene product are involved in the pathogenesis of BFNC. Thus, the presence of an altered (or a mutant) *KCNQ2* or *KCNQ3* gene which produces a protein having a loss of function, or altered function, directly causes BFNC which increases the risk of seizures. In order to detect a *KCNQ2* or *KCNQ3* gene mutation, a biological sample is prepared and analyzed for a difference between the sequence of the allele being analyzed and the sequence of the wild-type allele. Mutant *KCNQ2* or *KCNQ3* alleles can be initially identified by any of the techniques described above. The mutant alleles are then sequenced to identify the specific mutation of the particular mutant allele. Alternatively, mutant alleles can be initially identified by identifying mutant (altered) proteins, using conventional techniques. The mutant alleles are then sequenced to identify the specific mutation for each allele. The mutations, especially those which lead to an altered function of the protein, are then used for the diagnostic and prognostic methods of the present invention.

This is the first human idiopathic generalized epilepsy for which a  $K^+$  channel has been implicated. BFNC is considered to be a true idiopathic epilepsy without the degenerative characteristics associated with other syndromes such as progressive myoclonus epilepsy of the Unverricht-Lundborg type. It is not surprising, therefore, that an alteration in a gene which directly regulates neuronal excitability causes this epileptic disorder. Voltage-gated potassium channels repolarize neuronal membranes that have been depolarized by  $Na^+$  and  $Ca^{++}$  voltage-gated ion channels.  $K^+$  channels are also thought to repolarize neuronal membranes following activation of excitatory neurotransmitter ion channels, including glutamate and acetylcholine. In the presence of mutant *KCNQ2* or *KCNQ3* channels with reduced function, excitatory ligand and voltage-gated channels that are activated would remain open for a longer duration (Keating and Sanguinetti, 1996; Meldrum, 1995; McNamara, 1994). Such unchecked activity of excitatory systems could lead to

an epileptic phenotype. Electrophysiologic analysis of the mutant KCNQ2 and KCNQ3 channels will shed light on how the mutations identified in the current study produce an epileptic phenotype. It is likely that KCNQ2 and KCNQ3 will have biophysical properties similar to the delayed rectifier KCNQ1 channel. KCNQ1 alpha subunits coassemble with minK beta subunits to form heteromultimeric  $I_{Ks}$  channels in the heart (Sanguinetti et al., 1996). It is possible that KCNQ2 and KCNQ3 subunits coassemble with minK-like beta subunits in the brain. This interaction may also alter the gating properties of the resulting heteromultimeric channel as is the case for KCNQ1.

Mutations in  $K^+$  channels have been associated with epilepsy in only one other case, the weaver mouse, where a single missense mutation in the GIRK2 gene produces spontaneous seizures (Patil et al., 1995; Signorini et al., 1997). Mutations in  $K^+$  channels have been implicated in other human disorders such as the Long QT syndrome on chromosome 11 and ataxia/myokymia on chromosome 12 (Wang et al., 1996; Neyroud et al., 1997; Russell et al., 1996; Chandy and Gutman, 1995; Browne et al., 1994). Long QT is associated with four loci, two of which are the  $K^+$  channel genes HERG and KCNQ1. In KCNQ1, mutational hot spots have been identified in the pore and S6 domains where missense mutations in these regions account for a majority of the disease causing mutations in LQT (Russell et al., 1996; Wang et al., 1996).

Since the first publications of the finding of the *KCNQ2* and *KCNQ3* genes, there have been several more publications. Iannotti et al. (1998) found that there are two splice variants of *KCNQ2*. These are a long and a short form which differ in their C-termini. The long form is expressed exclusively in human brain (adult and fetal), where it is restricted to neuronal rather than glial cells. The short form is expressed weakly in adult brain but is prominent in fetal brain and testes (Iannotti et al., 1998). Gribkoff et al. (1998) cloned and expressed a mouse homologue of *KCNQ2* in *Xenopus* oocytes and performed two-electrode voltage clamp studies. Dworetzky et al. (1998) cloned a mouse homologue of *KCNQ2* and also noted alternative splice variants in the 3' region of the gene. They also performed Northern blots and measured polarization in *Xenopus* oocytes expressing the mouse gene. Yang et al. (1998) have also cloned and expressed the human *KCNQ2* and *KCNQ3*. They note that the encoded proteins act like KCNQ1 in eliciting voltage-gated, rapidly activating  $K^+$ -selective currents, but in contrast to KCNQ1, the KCNQ2 and KCNQ3 protein induced currents are not augmented by coexpression of KCNE1. However, coexpression of KCNQ2 and KCNQ3 results in a substantial synergistic increase in current amplitude (Yang et al., 1998). Finally, Biervert et al. (1998) cloned human *KCNQ2* and expressed it in *Xenopus* oocytes.

### Definitions

The present invention employs the following definitions.

"**Amplification of Polynucleotides**" utilizes methods such as the polymerase chain reaction (PCR), ligation amplification (or ligase chain reaction, LCR) and amplification methods based on the use of Q-beta replicase. Also useful are strand displacement amplification (SDA), thermophilic SDA, and nucleic acid sequence based amplification (3SR or NASBA). These methods are well known and widely practiced in the art. See, e.g., U.S. Patents 4,683,195 and 4,683,202 and Innis et al., 1990 (for PCR); Wu and Wallace, 1989 (for LCR); U.S. Patents 5,270,184 and 5,455,166 and Walker et al., 1992 (for SDA); Spargo et al., 1996 (for thermophilic SDA) and U.S. Patent 5,409,818, Fahy et al., 1991 and Compton, 1991 for 3SR and NASBA. Reagents and hardware for conducting PCR are commercially available. Primers useful to amplify sequences from the *KCNQ2* or *KCNQ3* region are preferably complementary to, and hybridize specifically to sequences in the *KCNQ2* or *KCNQ3* region or in regions that flank a target region therein. *KCNQ2* or *KCNQ3* sequences generated by amplification may be sequenced directly. Alternatively, but less desirably, the amplified sequence(s) may be cloned prior to sequence analysis. A method for the direct cloning and sequence analysis of enzymatically amplified genomic segments has been described by Scharf et al., 1986.

"**Analyte polynucleotide**" and "**analyte strand**" refer to a single- or double-stranded polynucleotide which is suspected of containing a target sequence, and which may be present in a variety of types of samples, including biological samples.

"**Antibodies.**" The present invention also provides polyclonal and/or monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof, which are capable of specifically binding to the *KCNQ2* or *KCNQ3* polypeptide and fragments thereof or to polynucleotide sequences from the *KCNQ2* *KCNQ3* region. The term "**antibody**" is used both to refer to a homogeneous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Polypeptides may be prepared synthetically in a peptide synthesizer and coupled to a carrier molecule (e.g., keyhole limpet hemocyanin) and injected over several months into rabbits. Rabbit sera is tested for immunoreactivity to the *KCNQ2* or *KCNQ3* polypeptide or fragment. Monoclonal antibodies may be made by injecting mice with the protein polypeptides, fusion proteins or fragments thereof. Monoclonal antibodies will be screened by ELISA and tested for specific immunoreactivity with *KCNQ2* or *KCNQ3* polypeptide or fragments

thereof. See, Harlow and Lane, 1988. These antibodies will be useful in assays as well as pharmaceuticals.

Once a sufficient quantity of desired polypeptide has been obtained, it may be used for various purposes. A typical use is the production of antibodies specific for binding. These 5 antibodies may be either polyclonal or monoclonal, and may be produced by *in vitro* or *in vivo* techniques well known in the art. For production of polyclonal antibodies, an appropriate target immune system, typically mouse or rabbit, is selected. Substantially purified antigen is presented to the immune system in a fashion determined by methods appropriate for the animal and by other parameters well known to immunologists. Typical sites for injection are in footpads, 10 intramuscularly, intraperitoneally, or intradermally. Of course, other species may be substituted for mouse or rabbit. Polyclonal antibodies are then purified using techniques known in the art, adjusted for the desired specificity.

An immunological response is usually assayed with an immunoassay. Normally, such immunoassays involve some purification of a source of antigen, for example, that produced by the 15 same cells and in the same fashion as the antigen. A variety of immunoassay methods are well known in the art. See, e.g., Harlow and Lane, 1988, or Goding, 1986.

Monoclonal antibodies with affinities of  $10^{-8} \text{ M}^{-1}$  or preferably  $10^{-9}$  to  $10^{-10} \text{ M}^{-1}$  or stronger will typically be made by standard procedures as described, e.g., in Harlow and Lane, 1988 or Goding, 1986. Briefly, appropriate animals will be selected and the desired immunization protocol 20 followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the desired region of the antigen.

Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic 25 polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors. See Huse et al., 1989. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific 30 and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include U.S. Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345;

4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced (see U.S. Patent 4,816,567).

**"Binding partner"** refers to a molecule capable of binding a ligand molecule with high specificity, as for example, an antigen and an antigen-specific antibody or an enzyme and its inhibitor. In general, the specific binding partners must bind with sufficient affinity to immobilize the analyte copy/complementary strand duplex (in the case of polynucleotide hybridization) under the isolation conditions. Specific binding partners are known in the art and include, for example, biotin and avidin or streptavidin, IgG and protein A, the numerous, known receptor-ligand couples, and complementary polynucleotide strands. In the case of complementary polynucleotide binding partners, the partners are normally at least about 15 bases in length, and may be at least 40 bases in length. It is well recognized by those of skill in the art that lengths shorter than 15 (e.g., 8 bases), between 15 and 40, and greater than 40 bases may also be used. The polynucleotides may be composed of DNA, RNA, or synthetic nucleotide analogs. Further binding partners can be identified using, e.g., the two-hybrid yeast screening assay as described herein.

**A "biological sample"** refers to a sample of tissue or fluid suspected of containing an analyte polynucleotide or polypeptide from an individual including, but not limited to, e.g., plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, blood cells, tumors, organs, tissue and samples of *in vitro* cell culture constituents.

**"Encode"**. A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof. The anti-sense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

**"Isolated" or "substantially pure"**. An "isolated" or "substantially pure" nucleic acid (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native human sequence or protein, e.g., ribosomes, polymerases, many other human genome sequences and proteins. The term embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

"KCNQ2 Allele" refers to normal alleles of the KCNQ2 locus as well as alleles of KCNQ2 carrying variations that cause BFNC and/or rolandic epilepsy.

"KCNQ3 Allele" refers to normal alleles of the KCNQ3 locus as well as alleles of KCNQ3 carrying variations that cause BFNC and/or JME.

5 "KCNQ2 Locus", "KCNQ2 Gene", "KCNQ2 Nucleic Acids" or "KCNQ2 Polynucleotide" each refer to polynucleotides, all of which are in the *KCNQ2* region, that are likely to be expressed in normal tissue, certain alleles of which result in BFNC and/or rolandic epilepsy. The KCNQ2 locus is intended to include coding sequences, intervening sequences and regulatory elements controlling transcription and/or translation. The KCNQ2 locus is intended to include all  
10 allelic variations of the DNA sequence.

"KCNQ3 Locus", "KCNQ3 Gene", "KCNQ3 Nucleic Acids" or "KCNQ3 Polynucleotide" each refer to polynucleotides, all of which are in the *KCNQ3* region, that are likely to be expressed in normal tissue, certain alleles of which result in BFNC and/or JME. The KCNQ3 locus is intended to include coding sequences, intervening sequences and regulatory elements  
15 controlling transcription and/or translation. The KCNQ3 locus is intended to include all allelic variations of the DNA sequence.

These terms, when applied to a nucleic acid, refer to a nucleic acid which encodes a human KCNQ2 or KCNQ3 polypeptide, fragment, homolog or variant, including, e.g., protein fusions or deletions. The nucleic acids of the present invention will possess a sequence which is either derived  
20 from, or substantially similar to a natural KCNQ2- or KCNQ3-encoding gene or one having substantial homology with a natural KCNQ2- or KCNQ3-encoding gene or a portion thereof.

The *KCNQ2* or *KCNQ3* gene or nucleic acid includes normal alleles of the *KCNQ2* or *KCNQ3* gene, respectively, including silent alleles having no effect on the amino acid sequence of the KCNQ2 or KCNQ3 polypeptide as well as alleles leading to amino acid sequence variants of  
25 the KCNQ2 or KCNQ3 polypeptide that do not substantially affect its function. These terms also include alleles having one or more mutations which adversely affect the function of the KCNQ2 or KCNQ3 polypeptide. A mutation may be a change in the *KCNQ2* or *KCNQ3* nucleic acid sequence which produces a deleterious change in the amino acid sequence of the KCNQ2 or KCNQ3 polypeptide, resulting in partial or complete loss of KCNQ2 or KCNQ3 function, respectively, or  
30 may be a change in the nucleic acid sequence which results in the loss of effective KCNQ2 or KCNQ3 expression or the production of aberrant forms of the KCNQ2 or KCNQ3 polypeptide.

The *KCNQ2* or *KCNQ3* nucleic acid may be that shown in SEQ ID NO:1 (*KCNQ2*) or SEQ ID NO:6 (*KCNQ3*) or it may be an allele as described above or a variant or derivative differing from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to the nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

Thus, nucleic acid according to the present invention may include a sequence different from the sequence shown in SEQ ID NOs:1 and 6 yet encode a polypeptide with the same amino acid sequence as shown in SEQ ID NOs:2 (*KCNQ2*) and 7 (*KCNQ3*). That is, nucleic acids of the present invention include sequences which are degenerate as a result of the genetic code. On the other hand, the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in SEQ ID NOs:2 and 7. Nucleic acid encoding a polypeptide which is an amino acid sequence variant, derivative or allele of the amino acid sequence shown in SEQ ID NOs:2 and 7 is also provided by the present invention.

The *KCNQ2* or *KCNQ3* gene, respectively, also refers to (a) any DNA sequence that (i) hybridizes to the complement of the DNA sequences that encode the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:6 under highly stringent conditions (Ausubel et al., 1992) and (ii) encodes a gene product functionally equivalent to *KCNQ2* or *KCNQ3*, or (b) any DNA sequence that (i) hybridizes to the complement of the DNA sequences that encode the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:7 under less stringent conditions, such as moderately stringent conditions (Ausubel et al., 1992) and (ii) encodes a gene product functionally equivalent to *KCNQ2* or *KCNQ3*. The invention also includes nucleic acid molecules that are the complements of the sequences described herein.

The polynucleotide compositions of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a

designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The present invention provides recombinant nucleic acids comprising all or part of the  
5 *KCNQ2* or *KCNQ3* region. The recombinant construct may be capable of replicating autonomously in a host cell. Alternatively, the recombinant construct may become integrated into the chromosomal DNA of the host cell. Such a recombinant polynucleotide comprises a polynucleotide of genomic, cDNA, semi-synthetic, or synthetic origin which, by virtue of its origin or manipulation, 1) is not associated with all or a portion of a polynucleotide with which it is associated in nature; 2) is linked  
10 to a polynucleotide other than that to which it is linked in nature; or 3) does not occur in nature. Where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

Therefore, recombinant nucleic acids comprising sequences otherwise not naturally occurring are provided by this invention. Although the wild-type sequence may be employed, it will  
15 often be altered, e.g., by deletion, substitution or insertion. cDNA or genomic libraries of various types may be screened as natural sources of the nucleic acids of the present invention, or such nucleic acids may be provided by amplification of sequences resident in genomic DNA or other natural sources, e.g., by PCR. The choice of cDNA libraries normally corresponds to a tissue source which is abundant in mRNA for the desired proteins. Phage libraries are normally preferred, but  
20 other types of libraries may be used. Clones of a library are spread onto plates, transferred to a substrate for screening, denatured and probed for the presence of desired sequences.

The DNA sequences used in this invention will usually comprise at least about five codons (15 nucleotides), more usually at least about 7-15 codons, and most preferably, at least about 35 codons. One or more introns may also be present. This number of nucleotides is usually about the  
25 minimal length required for a successful probe that would hybridize specifically with a *KCNQ2*- or *KCNQ3*-encoding sequence. In this context, oligomers of as low as 8 nucleotides, more generally 8-17 nucleotides, can be used for probes, especially in connection with chip technology.

Techniques for nucleic acid manipulation are described generally, for example, in Sambrook et al., 1989 or Ausubel et al., 1992. Reagents useful in applying such techniques, such as restriction  
30 enzymes and the like, are widely known in the art and commercially available from such vendors as New England BioLabs, Boehringer Mannheim, Amersham, Promega, U. S. Biochemicals, New England Nuclear, and a number of other sources. The recombinant nucleic acid sequences used to



produce fusion proteins of the present invention may be derived from natural or synthetic sequences. Many natural gene sequences are obtainable from various cDNA or from genomic libraries using appropriate probes. See, GenBank, National Institutes of Health.

As used herein, a "**portion**" of the *KCNQ2* or *KCNQ3* locus or region or allele is defined  
5 as having a minimal size of at least about eight nucleotides, or preferably about 15 nucleotides, or more preferably at least about 25 nucleotides, and may have a minimal size of at least about 40 nucleotides. This definition includes all sizes in the range of 8-40 nucleotides as well as greater than 40 nucleotides. Thus, this definition includes nucleic acids of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400, 500 nucleotides, or nucleic acids having any number of nucleotides within these ranges  
10 of values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc., nucleotides), or nucleic acids having more than 500 nucleotides. The present invention includes all novel nucleic acids having at least 8 nucleotides derived from SEQ ID NO:1 or SEQ ID NO:6, its complement or functionally equivalent nucleic acid sequences. The present invention does not include nucleic acids which exist in the prior art. That is, the present invention includes all nucleic acids having at least 8 nucleotides derived  
15 from SEQ ID NO:1 or SEQ ID NO:6 with the proviso that it does not include nucleic acids existing in the prior art.

"**KCNQ2 protein**" or "**KCNQ2 polypeptide**" refers to a protein or polypeptide encoded by the *KCNQ2* locus, variants or fragments thereof. The term "polypeptide" refers to a polymer of amino acids and its equivalent and does not refer to a specific length of the product; thus, peptides,  
20 oligopeptides and proteins are included within the definition of a polypeptide. This term also does not refer to, or exclude modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages as well as other modifications known in the art, both  
25 naturally and non-naturally occurring. Ordinarily, such polypeptides will be at least about 50% homologous to the native *KCNQ2* sequence, preferably in excess of about 90%, and more preferably at least about 95% homologous. Also included are proteins encoded by DNA which hybridize under high or low stringency conditions, to *KCNQ2*-encoding nucleic acids and closely related polypeptides or proteins retrieved by antisera to the *KCNQ2* protein(s).

30 "**KCNQ3 protein**" or "**KCNQ3 polypeptide**" refers to a protein or polypeptide encoded by the *KCNQ3* locus, variants or fragments thereof. The term "polypeptide" refers to a polymer of amino acids and its equivalent and does not refer to a specific length of the product; thus, peptides,

oligopeptides and proteins are included within the definition of a polypeptide. This term also does not refer to, or exclude modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages as well as other modifications known in the art, both naturally and non-naturally occurring. Ordinarily, such polypeptides will be at least about 50% homologous to the native KCNQ3 sequence, preferably in excess of about 90%, and more preferably at least about 95% homologous. Also included are proteins encoded by DNA which hybridize under high or low stringency conditions, to KCNQ3-encoding nucleic acids and closely related polypeptides or proteins retrieved by antisera to the KCNQ3 protein(s).

The KCNQ2 or KCNQ3 polypeptide may be that shown in SEQ ID NO:2 or SEQ ID NO:7 which may be in isolated and/or purified form, free or substantially free of material with which it is naturally associated. The polypeptide may, if produced by expression in a prokaryotic cell or produced synthetically, lack native post-translational processing, such as glycosylation. Alternatively, the present invention is also directed to polypeptides which are sequence variants, alleles or derivatives of the KCNQ2 or KCNQ3 polypeptide. Such polypeptides may have an amino acid sequence which differs from that set forth in SEQ ID NO:2 or SEQ ID NO:7 by one or more of addition, substitution, deletion or insertion of one or more amino acids. Preferred such polypeptides have KCNQ2 or KCNQ3 function.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, such as stability against proteolytic cleavage, without the loss of other functions or properties. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. Preferred substitutions are ones which are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and tyrosine, phenylalanine.

Certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules or binding sites on proteins interacting

with the KCNQ2 or KCNQ3 polypeptide. Since it is the interactive capacity and nature of a protein which defines that protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydrophobic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle, 1982). Alternatively, the substitution of like amino acids can be made effectively on the basis of hydrophilicity. The importance of hydrophilicity in conferring interactive biological function of a protein is generally understood in the art (U.S. Patent 4,554,101). The use of the hydrophobic index or hydrophilicity in designing polypeptides is further discussed in U.S. Patent 5,691,198.

The length of polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues.

**"Operably linked"** refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression.

The term **peptide mimetic** or **mimetic** is intended to refer to a substance which has the essential biological activity of the KCNQ2 or KCNQ3 polypeptide. A peptide mimetic may be a peptide-containing molecule that mimics elements of protein secondary structure (Johnson et al., 1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen, enzyme and substrate or scaffolding proteins. A peptide mimetic is designed to permit molecular interactions similar to the natural molecule. A mimetic may not be a peptide at all, but it will retain the essential biological activity of natural KCNQ2 or KCNQ3 polypeptide.

**"Probes"**. Polynucleotide polymorphisms associated with *KCNQ2* or *KCNQ3* alleles which predispose to BFNC, rolandic epilepsy or JME are detected by hybridization with a polynucleotide probe which forms a stable hybrid with that of the target sequence, under highly stringent to moderately stringent hybridization and wash conditions. If it is expected that the probes will be perfectly complementary to the target sequence, high stringency conditions will be used. Hybridization stringency may be lessened if some mismatching is expected, for example, if variants are expected with the result that the probe will not be completely complementary. Conditions are

chosen which rule out nonspecific/adventitious bindings, that is, which minimize noise. (It should be noted that throughout this disclosure, if it is simply stated that "stringent" conditions are used that is meant to be read as "high stringency" conditions are used.) Since such indications identify neutral DNA polymorphisms as well as mutations, these indications need further analysis to demonstrate  
5 detection of a *KCNQ2* or *KCNQ3* susceptibility allele.

Probes for *KCNQ2* alleles may be derived from the sequences of the *KCNQ2* region, its cDNA, functionally equivalent sequences, or the complements thereof. Probes for *KCNQ3* alleles may be derived from the sequences of the *KCNQ3* region, its cDNA, functionally equivalent sequences, or the complements thereof. The probes may be of any suitable length, which span all  
10 or a portion of the *KCNQ2* or *KCNQ3* region, and which allow specific hybridization to the region. If the target sequence contains a sequence identical to that of the probe, the probes may be short, e.g., in the range of about 8-30 base pairs, since the hybrid will be relatively stable under even highly stringent conditions. If some degree of mismatch is expected with the probe, i.e., if it is suspected that the probe will hybridize to a variant region, a longer probe may be employed which  
15 hybridizes to the target sequence with the requisite specificity.

The probes will include an isolated polynucleotide attached to a label or reporter molecule and may be used to isolate other polynucleotide sequences, having sequence similarity by standard methods. For techniques for preparing and labeling probes see, e.g., Sambrook et al., 1989 or Ausubel et al., 1992. Other similar polynucleotides may be selected by using homologous  
20 polynucleotides. Alternatively, polynucleotides encoding these or similar polypeptides may be synthesized or selected by use of the redundancy in the genetic code. Various codon substitutions may be introduced, e.g., by silent changes (thereby producing various restriction sites) or to optimize expression for a particular system. Mutations may be introduced to modify the properties of the polypeptide, perhaps to change the polypeptide degradation or turnover rate.

25 ~~Probes comprising synthetic oligonucleotides or other polynucleotides of the present invention may be derived from naturally occurring or recombinant single- or double-stranded polynucleotides, or be chemically synthesized. Probes may also be labeled by nick translation, Klenow fill-in reaction, or other methods known in the art.~~

Portions of the polynucleotide sequence having at least about eight nucleotides, usually at  
30 least about 15 nucleotides, and fewer than about 9 kb, usually fewer than about 1.0 kb, from a polynucleotide sequence encoding *KCNQ2* or *KCNQ3* are preferred as probes. This definition therefore includes probes of sizes 8 nucleotides through 9000 nucleotides. Thus, this definition

includes probes of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400 or 500 nucleotides or probes having any number of nucleotides within these ranges of values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc., nucleotides), or probes having more than 500 nucleotides. The probes may also be used to determine whether mRNA encoding *KCNQ2* or *KCNQ3* is present in a cell or tissue. The present invention includes all novel probes having at least 8 nucleotides derived from SEQ ID NO:1 or SEQ ID NO:6, its complement or functionally equivalent nucleic acid sequences. The present invention does not include probes which exist in the prior art. That is, the present invention includes all probes having at least 8 nucleotides derived from SEQ ID NO:1 or SEQ ID NO:6 with the proviso that they do not include probes existing in the prior art.

Similar considerations and nucleotide lengths are also applicable to primers which may be used for the amplification of all or part of the *KCNQ2* or *KCNQ3* gene. Thus, a definition for primers includes primers of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400, 500 nucleotides, or primers having any number of nucleotides within these ranges of values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc. nucleotides), or primers having more than 500 nucleotides, or any number of nucleotides between 500 and 9000. The primers may also be used to determine whether mRNA encoding *KCNQ2* or *KCNQ3* is present in a cell or tissue. The present invention includes all novel primers having at least 8 nucleotides derived from the *KCNQ2* or *KCNQ3* locus for amplifying the *KCNQ2* or *KCNQ3* gene, its complement or functionally equivalent nucleic acid sequences. The present invention does not include primers which exist in the prior art. That is, the present invention includes all primers having at least 8 nucleotides with the proviso that it does not include primers existing in the prior art.

**"Protein modifications or fragments"** are provided by the present invention for *KCNQ2* or *KCNQ3* polypeptides or fragments thereof which are substantially homologous to primary structural sequence but which include, e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, e.g., with radionuclides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as <sup>32</sup>P, ligands which bind to labeled antiligands (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability

requirements, and available instrumentation. Methods of labeling polypeptides are well known in the art. See Sambrook et al., 1989 or Ausubel et al., 1992.

Besides substantially full-length polypeptides, the present invention provides for biologically active fragments of the polypeptides. Significant biological activities include ligand-binding, immunological activity and other biological activities characteristic of KCNQ2 or KCNQ3 polypeptides. Immunological activities include both immunogenic function in a target immune system, as well as sharing of immunological epitopes for binding, serving as either a competitor or substitute antigen for an epitope of the KCNQ2 or KCNQ3 protein. As used herein, "epitope" refers to an antigenic determinant of a polypeptide. An epitope could comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods of determining the spatial conformation of such amino acids are known in the art.

For immunological purposes, tandem-repeat polypeptide segments may be used as immunogens, thereby producing highly antigenic proteins. Alternatively, such polypeptides will serve as highly efficient competitors for specific binding. Production of antibodies specific for KCNQ2 or KCNQ3 polypeptides or fragments thereof is described below.

The present invention also provides for fusion polypeptides, comprising KCNQ2 or KCNQ3 polypeptides and fragments. Homologous polypeptides may be fusions between two or more KCNQ2 or KCNQ3 polypeptide sequences or between the sequences of KCNQ2 or KCNQ3 and a related protein. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. For example, ligand-binding or other domains may be "swapped" between different new fusion polypeptides or fragments. Such homologous or heterologous fusion polypeptides may display, for example, altered strength or specificity of binding. Fusion partners include immunoglobulins, bacterial  $\beta$ -galactosidase, trpE, protein A,  $\beta$ -lactamase, alpha amylase, alcohol dehydrogenase and yeast alpha mating factor. See Godowski et al., 1988.

Fusion proteins will typically be made by either recombinant nucleic acid methods, as described below, or may be chemically synthesized. Techniques for the synthesis of polypeptides are described, for example, in Merrifield, 1963.

**"Protein purification"** refers to various methods for the isolation of the KCNQ2 or KCNQ3 polypeptides from other biological material, such as from cells transformed with recombinant nucleic acids encoding KCNQ2 or KCNQ3, and are well known in the art. For example, such

polypeptides may be purified by immunoaffinity chromatography employing, e.g., the antibodies provided by the present invention. Various methods of protein purification are well known in the art, and include those described in Deutscher, 1990 and Scopes, 1982.

The terms "isolated", "substantially pure", and "substantially homogeneous" are used interchangeably to describe a protein or polypeptide which has been separated from components which accompany it in its natural state. A monomeric protein is substantially pure when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art which are utilized for purification.

A KCNQ2 or KCNQ3 protein is substantially free of naturally associated components when it is separated from the native contaminants which accompany it in its natural state. Thus, a polypeptide which is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

A polypeptide produced as an expression product of an isolated and manipulated genetic sequence is an "isolated polypeptide," as used herein, even if expressed in a homologous cell type. Synthetically made forms or molecules expressed by heterologous cells are inherently isolated molecules.

**"Recombinant nucleic acid"** is a nucleic acid which is not naturally occurring, or which is made by the artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions.

**"Regulatory sequences"** refers to those sequences normally within 100 kb of the coding region of a locus, but they may also be more distant from the coding region, which affect the expression of the gene (including transcription of the gene, and translation, splicing, stability or the like of the messenger RNA).

5       **"Substantial homology or similarity"**. A nucleic acid or fragment thereof is "substantially homologous" ("or substantially similar") to another if, when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least  
10   about 95-98% of the nucleotide bases.

To determine homology between two different nucleic acids, the percent homology is to be determined using the BLASTN program "BLAST 2 sequences". This program is available for public use from the National Center for Biotechnology Information (NCBI) over the Internet (<http://www.ncbi.nlm.nih.gov/gorf/bl2.html>) (Altschul et al., 1997). The parameters to be used are  
15   whatever combination of the following yields the highest calculated percent homology (as calculated below) with the default parameters shown in parentheses:

Program - blastn

Matrix - 0 BLOSUM62

Reward for a match - 0 or 1 (1)

20   Penalty for a mismatch - 0, -1, -2 or -3 (-2)

Open gap penalty - 0, 1, 2, 3, 4 or 5 (5)

Extension gap penalty - 0 or 1 (1)

Gap x\_dropoff - 0 or 50 (50)

Expect - 10

25   Along with a variety of other results, this program shows a percent identity across the complete strands or across regions of the two nucleic acids being matched. The program shows as part of the results an alignment and identity of the two strands being compared. If the strands are of equal length then the identity will be calculated across the complete length of the nucleic acids. If the strands are of unequal lengths, then the length of the shorter nucleic acid is to be used. If the  
30   nucleic acids are quite similar across a portion of their sequences but different across the rest of their sequences, the blastn program "BLAST 2 Sequences" will show an identity across only the similar portions, and these portions are reported individually. For purposes of determining homology



herein, the percent homology refers to the shorter of the two sequences being compared. If any one region is shown in different alignments with differing percent identities, the alignments which yield the greatest homology are to be used. The averaging is to be performed as in this example of SEQ ID NOs:20 and 21.

5 5'-ACCGTAGCTACGTACGTATATAGAAAGGGCGCGATCGTCGTCGCGTATGACGAC  
TTAGCATGC-3' (SEQ ID NO:20)

5'-ACCGGTAGCTACGTACGTTATTTAGAAAGGGGTGTGTGTGTGTGTGTAAACCGGG  
GTTTTTCGGGATCGTCCGTCGCGTATGACGACTTAGCCATGCACGGTATATCGTATTA  
GGACTAGCGATTGACTAG-3' (SEQ ID NO:21)

10 The program "BLAST 2 Sequences" shows differing alignments of these two nucleic acids depending upon the parameters which are selected. As examples, four sets of parameters were selected for comparing SEQ ID NOs:20 and 21 (gap x\_dropoff was 50 for all cases), with the results shown in Table 1. It is to be noted that none of the sets of parameters selected as shown in Table 1 is necessarily the best set of parameters for comparing these sequences. The percent homology  
15 is calculated by multiplying for each region showing identity the fraction of bases of the shorter strand within a region times the percent identity for that region and adding all of these together. For example, using the first set of parameters shown in Table 1, SEQ ID NO:20 is the short sequence (63 bases), and two regions of identity are shown, the first encompassing bases 4-29 (26 bases) of SEQ ID NO:20 with 92% identity to SEQ ID NO:21 and the second encompassing bases 39-59 (21  
20 bases) of SEQ ID NO:20 with 100% identity to SEQ ID NO:21. Bases 1-3, 30-38 and 60-63 (16 bases) are not shown as having any identity with SEQ ID NO:21. Percent homology is calculated as:  $(26/63)(92) + (21/63)(100) + (16/63)(0) = 71.3\%$  homology. The percents of homology calculated using each of the four sets of parameters shown are listed in Table 1. Several other combinations of parameters are possible, but they are not listed for the sake of brevity. It is seen  
25 that each set of parameters resulted in a different calculated percent homology. Because the result yielding the highest percent homology is to be used, based solely on these four sets of parameters one would state that SEQ ID NOs:20 and 21 have 87.1% homology. Again it is to be noted that use of other parameters may show an even higher homology for SEQ ID NOs:20 and 21, but for brevity not all the possible results are shown.

30 Alternatively, substantial homology or (similarity) exists when a nucleic acid or fragment thereof will hybridize to another nucleic acid (or a complementary strand thereof) under selective hybridization conditions, to a strand, or to its complement. Selectivity of hybridization exists when

TABLE 1

Parameter Values				Regions of identity (%)		Homology
Match	Mismatch	Open Gap	Extension Gap			
1	-2	5	1	4-29 of 20 and 5-31 of 21 (92%)	39-59 of 20 and 71-91 of 21 (100%)	71.3
1	-2	2	1	4-29 of 20 and 5-31 of 21 (92%)	33-63 of 20 and 64-96 of 21 (93%)	83.7
1	-1	5	1	-----	30-59 of 20 and 61-91 of 21 (93%)	44.3
1	-1	2	1	4-29 of 20 and 5-31 of 21 (92%)	30-63 of 20 and 61-96 of 21 (91%)	87.1

hybridization which is substantially more selective than total lack of specificity occurs. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, Kanehisa, 1984. The length of homology comparison, as  
5 described, may be over longer stretches, and in certain embodiments will often be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides.

Nucleic acid hybridization will be affected by such conditions as salt concentration,  
10 temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than  
15 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. The stringency conditions are dependent on the length of the nucleic acid and the base composition of the nucleic acid and can be determined by techniques well known in the art. See, e.g., Wetmur and Davidson, 1968.

Probe sequences may also hybridize specifically to duplex DNA under certain conditions  
20 to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art.

The terms "**substantial homology**" or "**substantial identity**", when referring to polypeptides, indicate that the polypeptide or protein in question exhibits at least about 30% identity with an entire naturally-occurring protein or a portion thereof, usually at least about 70% identity,  
25 more usually at least about 80% identity, preferably at least about 90% identity, and more preferably at least about 95% identity.

Homology, for polypeptides, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wisconsin 53705. Protein  
30 analysis software matches similar sequences using measures of homology assigned to various substitutions, deletions and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine: valine, isoleucine, leucine: aspartic acid,

glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

"**Substantially similar function**" refers to the function of a modified nucleic acid or a modified protein, with reference to the wild-type *KCNQ2* or *KCNQ3* nucleic acid or wild-type  
5 *KCNQ2* or *KCNQ3* polypeptide. The modified polypeptide will be substantially homologous to the wild-type *KCNQ2* or *KCNQ3* polypeptide and will have substantially the same function. The modified polypeptide may have an altered amino acid sequence and/or may contain modified amino acids. In addition to the similarity of function, the modified polypeptide may have other useful properties, such as a longer half-life. The similarity of function (activity) of the modified  
10 polypeptide may be substantially the same as the activity of the wild-type *KCNQ2* or *KCNQ3* polypeptide. Alternatively, the similarity of function (activity) of the modified polypeptide may be higher than the activity of the wild-type *KCNQ2* or *KCNQ3* polypeptide. The modified polypeptide is synthesized using conventional techniques, or is encoded by a modified nucleic acid and produced using conventional techniques. The modified nucleic acid is prepared by conventional techniques.  
15 A nucleic acid with a function substantially similar to the wild-type *KCNQ2* or *KCNQ3* gene function produces the modified protein described above.

A polypeptide "**fragment**," "**portion**" or "**segment**" is a stretch of amino acid residues of at least about five to seven contiguous amino acids, often at least about seven to nine contiguous amino acids, typically at least about nine to 13 contiguous amino acids and, most preferably, at least  
20 about 20 to 30 or more contiguous amino acids.

The polypeptides of the present invention, if soluble, may be coupled to a solid-phase support, e.g., nitrocellulose, nylon, column packing materials (e.g., Sepharose beads), magnetic beads, glass wool, plastic, metal, polymer gels, cells, or other substrates. Such supports may take the form, for example, of beads, wells, dipsticks, or membranes.

25 "**Target region**" refers to a region of the nucleic acid which is amplified and/or detected. The term "**target sequence**" refers to a sequence with which a probe or primer will form a stable hybrid under desired conditions.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, and  
30 immunology. See, e.g., Maniatis et al., 1982; Sambrook et al., 1989; Ausubel et al., 1992; Glover, 1985; Anand, 1992; Guthrie and Fink, 1991. A general discussion of techniques and materials for

human gene mapping, including mapping of human chromosome 1, is provided, e.g., in White and Lalouel. 1988.

Preparation of recombinant or chemically synthesized  
5 nucleic acids: vectors, transformation, host cells

Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natural or synthetic polynucleotide fragments coding for a desired fragment will be incorporated into recombinant polynucleotide constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the  
10 polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cell lines. The purification of nucleic acids produced by the methods of the present invention are described, e.g., in Sambrook et al., 1989 or Ausubel et al., 1992.

15 The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Carruthers (1981) or the triester method according to Matteucci and Caruthers (1981), and may be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and  
20 annealing the strands together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and  
25 translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Such vectors may be  
30 prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Sambrook et al., 1989 or Ausubel et al., 1992.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may include, when appropriate, those naturally associated with the *KCNQ2* or *KCNQ3* gene. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al., 1989 or Ausubel et al., 1992; see also, e.g., Metzger et al., 1988.

5 Many useful vectors are known in the art and may be obtained from such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. Promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, 10 enzymes responsible for maltose and galactose utilization, and others. Vectors and promoters suitable for use in yeast expression are further described in Hitzeman et al., EP 73,675A. Appropriate non-native mammalian promoters might include the early and late promoters from SV40 (Fiers et al., 1978) or promoters derived from murine Molony leukemia virus, mouse tumor virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. Insect promoters 15 may be derived from baculovirus. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also Enhancers and Eukaryotic Gene Expression, Cold Spring Harbor Press, Cold Spring Harbor, New York (1983). See also, e.g., U.S. Patent Nos. 5,691,198; 5,735,500; 5,747,469 and 5,436,146.

20 While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells which express the inserts. Typical selection 25 genes encode proteins that a) confer resistance to antibiotics or other toxic substances, e.g. ampicillin, neomycin, methotrexate, etc., b) complement auxotrophic deficiencies, or c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art.

30 The vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al., 1988), or the vectors can be introduced directly into host cells by methods well known in the

art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome); and other methods. See generally, Sambrook et al., 1989 and  
5 Ausubel et al., 1992. The introduction of the polynucleotides into the host cell by any method known in the art, including, *inter alia*, those described above, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

Large quantities of the nucleic acids and polypeptides of the present invention may be  
10 prepared by expressing the *KCNQ2* or *KCNQ3* nucleic acid or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used.

Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant,  
15 insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. Propagation of mammalian cells in culture is *per se* well known. See, Jakoby and Pastan (eds.) (1979). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be appropriate, e.g., to  
20 provide higher expression, desirable glycosylation patterns, or other features. An example of a commonly used insect cell line is SF9.

Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline  
25 or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

Prokaryotic or eukaryotic cells transformed with the polynucleotides of the present invention will be useful not only for the production of the nucleic acids and polypeptides of the present invention, but also, for example, in studying the characteristics of *KCNQ2* or *KCNQ3* polypeptide.

30 The probes and primers based on the *KCNQ2* or *KCNQ3* gene sequence disclosed herein are used to identify homologous *KCNQ2* or *KCNQ3* gene sequences and proteins in other species.

These gene sequences and proteins are used in the diagnostic/prognostic, therapeutic and drug screening methods described herein for the species from which they have been isolated.

#### Methods of Use: Drug Screening

5 This invention is particularly useful for screening compounds by using the KCNQ2 or KCNQ3 polypeptide or binding fragment thereof in any of a variety of drug screening techniques.

The KCNQ2 or KCNQ3 polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, or borne on a cell surface. One method of drug screening utilizes eucaryotic or procaryotic host cells which are stably transformed with recombinant  
10 polynucleotides expressing the polypeptide or fragment, preferably in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, for the formation of complexes between a KCNQ2 or KCNQ3 polypeptide or fragment and the agent being tested, or examine the degree to which the formation of a complex between a KCNQ2 or KCNQ3 polypeptide or fragment and a known ligand is interfered with by the  
15 agent being tested.

Thus, the present invention provides methods of screening for drugs comprising contacting such an agent with a KCNQ2 or KCNQ3 polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the KCNQ2 or KCNQ3 polypeptide or fragment, or (ii) for the presence of a complex between the KCNQ2 or KCNQ3 polypeptide or fragment and a  
20 ligand, by methods well known in the art. In such competitive binding assays the KCNQ2 or KCNQ3 polypeptide or fragment is typically labeled. Free KCNQ2 or KCNQ3 polypeptide or fragment is separated from that present in a protein:protein complex, and the amount of free (i.e., uncomplexed) label is a measure of the binding of the agent being tested to KCNQ2 or KCNQ3 or its interference with KCNQ2(or KCNQ3):ligand binding, respectively. One may also measure the  
25 amount of bound, rather than free, KCNQ2 or KCNQ3. It is also possible to label the ligand rather than the KCNQ2 or KCNQ3 and to measure the amount of ligand binding to KCNQ2 or KCNQ3 in the presence and in the absence of the drug being tested.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the KCNQ2 or KCNQ3 polypeptides and is described in detail  
30 in Geysen (published PCT published application WO 84/03564). Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with KCNQ2 or KCNQ3 polypeptide



and washed. Bound KCNQ2 or KCNQ3 polypeptide is then detected by methods well known in the art.

Purified KCNQ2 or KCNQ3 can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to the polypeptide can be used  
5 to capture antibodies to immobilize the KCNQ2 or KCNQ3 polypeptide on the solid phase.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of specifically binding the KCNQ2 or KCNQ3 polypeptide compete with a test compound for binding to the KCNQ2 or KCNQ3 polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or  
10 more antigenic determinants of the KCNQ2 or KCNQ3 polypeptide.

The invention is particularly useful for screening compounds by using KCNQ2 or KCNQ3 protein in transformed cells, transfected oocytes or transgenic animals. The drug is added to the cells in culture or administered to a transgenic animal containing mutant KCNQ2 or KCNQ3 and the effect on the current of the potassium channel is compared to the current of a cell or animal  
15 containing the wild-type KCNQ2 or KCNQ3. Drug candidates which alter the current to a more normal level are useful for treating or preventing BFNC, rolandic epilepsy and JME.

The above screening methods are not limited to assays employing only KCNQ2 or KCNQ3 but are also applicable to studying KCNQ2- or KCNQ3-protein complexes. The effect of drugs on the activity of this complex is analyzed.

20 In accordance with these methods, the following assays are examples of assays which can be used for screening for drug candidates.

A mutant KCNQ2 or KCNQ3 (*per se* or as part of a fusion protein) is mixed with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type KCNQ2 or KCNQ3 binds. This mixing is performed in both the presence of a drug and the absence of the drug, and the amount  
25 of binding of the mutant KCNQ2 or KCNQ3 with the wild-type protein is measured. If the amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating BFNC, rolandic epilepsy or JME resulting from a mutation in *KCNQ2* or *KCNQ3*.

A wild-type KCNQ2 or KCNQ3 (*per se* or as part of a fusion protein) is mixed with a wild-  
30 type protein (*per se* or as part of a fusion protein) to which wild-type KCNQ2 or KCNQ3 binds. This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the wild-type KCNQ2 or KCNQ3 with the wild-type protein is measured. If the

amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating BFNC, rolandic epilepsy or JME resulting from a mutation in *KCNQ2* or *KCNQ3*.

A mutant protein, which as a wild-type protein binds to *KCNQ2* or *KCNQ3* (*per se* or as  
5 part of a fusion protein) is mixed with a wild-type *KCNQ2* or *KCNQ3* (*per se* or as part of a fusion protein). This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the mutant protein with the wild-type *KCNQ2* or *KCNQ3* is measured. If the amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating BFNC, rolandic epilepsy or JME resulting from a mutation  
10 in the gene encoding the protein.

The polypeptide of the invention may also be used for screening compounds developed as a result of combinatorial library technology. Combinatorial library technology provides an efficient way of testing a potential vast number of different substances for ability to modulate activity of a polypeptide. Such libraries and their use are known in the art. The use of peptide libraries is  
15 preferred. See, for example, WO 97/02048.

Briefly, a method of screening for a substance which modulates activity of a polypeptide may include contacting one or more test substances with the polypeptide in a suitable reaction medium, testing the activity of the treated polypeptide and comparing that activity with the activity of the polypeptide in comparable reaction medium untreated with the test substance or substances.  
20 A difference in activity between the treated and untreated polypeptides is indicative of a modulating effect of the relevant test substance or substances.

Prior to or as well as being screened for modulation of activity, test substances may be screened for ability to interact with the polypeptide, e.g., in a yeast two-hybrid system (e.g., Bartel et al., 1993; Fields and Song, 1989; Chevray and Nathans, 1992; Lee et al., 1995). This system may  
25 be used as a coarse screen prior to testing a substance for actual ability to modulate activity of the polypeptide. Alternatively, the screen could be used to screen test substances for binding to a *KCNQ2* or *KCNQ3* specific binding partner, or to find mimetics of the *KCNQ2* or *KCNQ3* polypeptide.

Following identification of a substance which modulates or affects polypeptide activity, the  
30 substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e., manufacture or formulation, or a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

Thus, the present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of polypeptide activity, in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a method comprising administration of such a composition comprising  
5 such a substance, a method comprising administration of such a composition to a patient, e.g., for treatment (which may include preventative treatment) of BFNC, rolandic epilepsy or JME, use of such a substance in the manufacture of a composition for administration, e.g., for treatment of BFNC, rolandic epilepsy or JME. and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or  
10 carrier, and optionally other ingredients.

A substance identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use.

15 The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., pure peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic  
20 design, synthesis and testing is generally used to avoid randomly screening large numbers of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by  
25 systematically varying the amino acid residues in the peptide, e.g., by substituting each residue in turn. Alanine scans of peptide are commonly used to refine such peptide motifs. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g., stereochemistry, bonding, size and/or charge, using data from a range of sources,  
30 e.g., spectroscopic techniques, x-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted onto it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

#### Methods of Use: Nucleic Acid Diagnosis and Diagnostic Kits

In order to detect the presence of a *KCNQ2* or *KCNQ3* allele predisposing an individual to BFNC, rolandic epilepsy or JME, a biological sample such as blood is prepared and analyzed for the presence or absence of susceptibility alleles of *KCNQ2* or *KCNQ3*. In order to detect the presence of BFNC, rolandic epilepsy or JME, or as a prognostic indicator, a biological sample is prepared and analyzed for the presence or absence of mutant alleles of *KCNQ2* or *KCNQ3*. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic laboratories, or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

Initially, the screening method involves amplification of the relevant *KCNQ2* or *KCNQ3* sequences. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such screening methods include two-step label amplification methodologies that are well known in the art. Both PCR and non-PCR based screening strategies can detect target sequences with a high level of sensitivity.

The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is the polymerase chain reaction (PCR). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy

number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes.

When the probes are used to detect the presence of the target sequences the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence, e.g. denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region of human chromosome 20 for *KCNQ2* or to the targeted region of human chromosome 8 for *KCNQ3*. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. These factors are outlined in, for example, Maniatis et al., 1982 and Sambrook et al., 1989. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadruplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies, gold nanoparticles and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these

variations are reviewed in, e.g., Matthews and Kricka, 1988; Landegren et al., 1988; Mifflin, 1989; U.S. Patent 4,868,105; and in EPO Publication No. 225,807.

As noted above, non-PCR based screening assays are also contemplated in this invention. This procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output resulting in a  $10^3$ - $10^6$  increase in sensitivity. For an example relating to the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes, see Jablonski et al., 1986.

Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding *KCNQ2* or *KCNQ3*. Allele specific probes are also contemplated within the scope of this example and exemplary allele specific probes include probes encompassing the predisposing mutations of this disclosure.

In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. For methods for labeling nucleic acid probes according to this embodiment see Martin et al., 1990. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well known embodiment of this example is the biotin-avidin type of interactions. For methods for labeling nucleic acid probes and their use in biotin-avidin based assays see Rigby et al., 1977 and Nguyen et al., 1992.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes capable of detecting *KCNQ2* or *KCNQ3*. Thus, in one example to detect the presence of *KCNQ2* or *KCNQ3* in a cell sample, more than one probe complementary to the gene is employed and in particular the number of different probes is alternatively two, three, or five different nucleic acid probe sequences. In another example, to detect the presence of mutations in the *KCNQ2* or *KCNQ3* gene sequence in a patient,

more than one probe complementary to these genes is employed where the cocktail includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in *KCNQ2* or *KCNQ3*. In this embodiment, any number of probes can be used, and will preferably include probes corresponding to the major gene mutations identified as predisposing an individual to BFNC, rolandic epilepsy or JME.

#### Methods of Use: Peptide Diagnosis and Diagnostic Kits

The presence of BFNC, rolandic epilepsy or JME can also be detected on the basis of the alteration of wild-type *KCNQ2* or *KCNQ3* polypeptide. Such alterations can be determined by sequence analysis in accordance with conventional techniques. More preferably, antibodies (polyclonal or monoclonal) are used to detect differences in, or the absence of *KCNQ2* or *KCNQ3* peptides. Techniques for raising and purifying antibodies are well known in the art and any such techniques may be chosen to achieve the preparations claimed in this invention. In a preferred embodiment of the invention, antibodies will immunoprecipitate *KCNQ2* or *KCNQ3* proteins from solution as well as react with these proteins on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, antibodies will detect *KCNQ2* or *KCNQ3* proteins in paraffin or frozen tissue sections, using immunocytochemical techniques.

Preferred embodiments relating to methods for detecting *KCNQ2* or *KCNQ3* or their mutations include enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies. Exemplary sandwich assays are described by David et al., in U.S. Patent Nos. 4,376,110 and 4,486,530, hereby incorporated by reference.

#### Methods of Use: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide *in vivo*. See, e.g., Hodgson, 1991. In one approach, one first determines the three-dimensional structure of a protein of interest (e.g., *KCNQ2* or *KCNQ3* polypeptide) by x-ray crystallography, by computer modeling or most typically, by a combination of approaches. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous

proteins. An example of rational drug design is the development of HIV protease inhibitors (Erickson et al., 1990). In addition, peptides (e.g., KCNQ2 or KCNQ3 polypeptide) are analyzed by an alanine scan (Wells, 1991). In this technique, an amino acid residue is replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is  
5 analyzed in this manner to determine the important regions of the peptide.

It is also possible to isolate a target-specific antibody, selected by a functional assay, and then to solve its crystal structure. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody.  
10 As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacore.

Thus, one may design drugs which have, e.g., improved KCNQ2 or KCNQ3 polypeptide  
15 activity or stability or which act as inhibitors, agonists, antagonists, etc. of KCNQ2 or KCNQ3 polypeptide activity. By virtue of the availability of cloned *KCNQ2* and *KCNQ3* sequences, sufficient amounts of the KCNQ2 and KCNQ3 polypeptides may be made available to perform such analytical studies as x-ray crystallography. In addition, the knowledge of the KCNQ2 and KCNQ3 protein sequences provided herein will guide those employing computer modeling techniques in  
20 place of, or in addition to x-ray crystallography.

#### Methods of Use: Gene Therapy

According to the present invention, a method is also provided of supplying wild-type KCNQ2 or KCNQ3 function to a cell which carries a mutant *KCNQ2* or *KCNQ3* allele, respectively.  
25 Supplying such a function should allow normal functioning of the recipient cells. The wild-type gene or a part of the gene may be introduced into the cell in a vector such that the gene remains extrachromosomal. In such a situation, the gene will be expressed by the cell from the extrachromosomal location. More preferred is the situation where the wild-type gene or a part thereof is introduced into the mutant cell in such a way that it recombines with the endogenous  
30 mutant gene present in the cell. Such recombination requires a double recombination event which results in the correction of the gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector



may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation and viral transduction are known in the art, and the choice of method is within the competence of the practitioner.

As generally discussed above, the *KCNQ2* or *KCNQ3* gene or fragment, where applicable, 5 may be employed in gene therapy methods in order to increase the amount of the expression products of such gene in cells. It may also be useful to increase the level of expression of the *KCNQ2* or *KCNQ3* gene even in those persons in which the mutant gene is expressed at a "normal" level, but the gene product is not fully functional.

Gene therapy would be carried out according to generally accepted methods, for example, 10 as described by Friedman (1991) or Culver (1996). Cells from a patient would be first analyzed by the diagnostic methods described above, to ascertain the production of KCNQ2 and/or KCNQ3 polypeptide in the cells. A virus or plasmid vector (see further details below), containing a copy of the *KCNQ2* or *KCNQ3* gene linked to expression control elements and capable of replicating inside the cells, is prepared. The vector may be capable of replicating inside the cells. Alternatively, the 15 vector may be replication deficient and is replicated in helper cells for use in gene therapy. Suitable vectors are known, such as disclosed in U.S. Patent 5,252,479 and PCT published application WO 93/07282 and U.S. Patent Nos. 5,691,198; 5,747,469; 5,436,146 and 5,753,500. The vector is then injected into the patient. If the transfected gene is not permanently incorporated into the genome of each of the targeted cells, the treatment may have to be repeated periodically.

20 Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and nonviral transfer methods. A number of viruses have been used as gene transfer vectors or as the basis for repairing gene transfer vectors, including papovaviruses (e.g., SV40, Madzak et al., 1992), adenovirus (Berkner, 1992; Berkner et al., 1988; Gorziglia and Kapikian, 1992; Quantin et al., 1992; Rosenfeld et al., 1992; Wilkinson and 25 Akrigg, 1992; Stratford-Perricaudet et al., 1990; Schneider et al., 1998), vaccinia virus (Moss, 1992; Moss, 1996), adeno-associated virus (Muzyczka, 1992; Ohi et al., 1990; Russell and Hirata, 1998), herpesviruses including HSV and EBV (Margolskee, 1992; Johnson et al., 1992; Fink et al., 1992; Breakefield and Geller, 1987; Freese et al., 1990; Fink et al., 1996), lentiviruses (Naldini et al., 1996), Sindbis and Semliki Forest virus (Berglund et al., 1993), and retroviruses of avian 30 (Bandyopadhyay and Temin, 1984; Petropoulos et al., 1992), murine (Miller, 1992; Miller et al., 1985; Sorge et al., 1984; Mann and Baltimore, 1985; Miller et al., 1988), and human origin (Shimada et al., 1991; Helseth et al., 1990; Page et al., 1990; Buchschacher and Panganiban, 1992).

Most human gene therapy protocols have been based on disabled murine retroviruses, although adenovirus and adeno-associated virus are also being used.

Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham and van der Eb, 1973; Pellicer et al., 1980); mechanical techniques, for example microinjection (Anderson et al., 1980; Gordon et al., 1980; Brinster et al., 1981; Costantini and Lacy, 1981); membrane fusion-mediated transfer via liposomes (Felgner et al., 1987; Wang and Huang, 1989; Kaneda et al., 1989; Stewart et al., 1992; Nabel et al., 1990; Lim et al., 1991); and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990; Wu et al., 1991; Zenke et al., 1990; Wu et al., 1989; Wolff et al., 1991; Wagner et al., 1990; Wagner et al., 1991; Cotten et al., 1990; Curiel et al., 1992; Curiel et al., 1991). Viral-mediated gene transfer can be combined with direct *in vitro* gene transfer using liposome delivery, allowing one to direct the viral vectors to the tumor cells and not into the surrounding nondividing cells. Alternatively, the retroviral vector producer cell line can be injected into tumors (Culver et al., 1992). Injection of producer cells would then provide a continuous source of vector particles. This technique has been approved for use in humans with inoperable brain tumors.

In an approach which combines biological and physical gene transfer methods, plasmid DNA of any size is combined with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged. For other techniques for the delivery of adenovirus based vectors see Schneider et al. (1998) and U.S. Patent Nos. 5,691,198; 5,747,469; 5,436,146 and 5,753,500.

Liposome/DNA complexes have been shown to be capable of mediating direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer process is nonspecific, localized *in vivo* uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992).

Expression vectors in the context of gene therapy are meant to include those constructs containing sequences sufficient to express a polynucleotide that has been cloned therein. In viral expression vectors, the construct contains viral sequences sufficient to support packaging of the construct. If the polynucleotide encodes *KCNQ2* or *KCNQ3*, expression will produce *KCNQ2* or *KCNQ3*. If the polynucleotide encodes an antisense polynucleotide or a ribozyme, expression will produce the antisense polynucleotide or ribozyme. Thus in this context, expression does not require

that a protein product be synthesized. In addition to the polynucleotide cloned into the expression vector, the vector also contains a promoter functional in eukaryotic cells. The cloned polynucleotide sequence is under control of this promoter. Suitable eukaryotic promoters include those described above. The expression vector may also include sequences, such as selectable markers and other  
5 sequences described herein.

Gene transfer techniques which target DNA directly to brain tissue is preferred. Receptor-mediated gene transfer, for example, is accomplished by the conjugation of DNA (usually in the form of covalently closed supercoiled plasmid) to a protein ligand via polylysine. Ligands are chosen on the basis of the presence of the corresponding ligand receptors on the cell surface of the  
10 target cell/tissue type. These ligand-DNA conjugates can be injected directly into the blood if desired and are directed to the target tissue where receptor binding and internalization of the DNA-protein complex occurs. To overcome the problem of intracellular destruction of DNA, coinfection with adenovirus can be included to disrupt endosome function.

The therapy is as follows: patients who carry a *KCNQ2* or *KCNQ3* susceptibility allele are  
15 treated with a gene delivery vehicle such that some or all of their brain precursor cells receive at least one additional copy of a functional normal *KCNQ2* or *KCNQ3* allele, respectively. In this step, the treated individuals have reduced risk of BFNC, rolandic epilepsy and/or JME to the extent that the effect of the susceptible allele has been countered by the presence of the normal allele.

## 20 Methods of Use: Peptide Therapy

Peptides which have *KCNQ2* or *KCNQ3* activity can be supplied to cells which carry mutant or missing *KCNQ2* or *KCNQ3* alleles, respectively. Protein can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors. Alternatively, *KCNQ2* or *KCNQ3* polypeptide can be extracted from *KCNQ2*- or *KCNQ3*-producing mammalian cells.  
25 In addition, the techniques of synthetic chemistry can be employed to synthesize *KCNQ2* or *KCNQ3* protein. Any of such techniques can provide the preparation of the present invention which comprises the *KCNQ2* or *KCNQ3* protein. The preparation is substantially free of other human proteins. This is most readily accomplished by synthesis in a microorganism or *in vitro*.

Active *KCNQ2* or *KCNQ3* molecules can be introduced into cells by microinjection or by  
30 use of liposomes, for example. Alternatively, some active molecules may be taken up by cells, actively or by diffusion. Supply of molecules with *KCNQ2* or *KCNQ3* activity should lead to partial reversal of BFNC, rolandic epilepsy and/or JME. Other molecules with *KCNQ2* or *KCNQ3*

activity (for example, peptides, drugs or organic compounds) may also be used to effect such a reversal. Modified polypeptides having substantially similar function are also used for peptide therapy.

## 5 Methods of Use: Transformed Hosts

Animals for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant *KCNQ2* and/or *KCNQ3* alleles, usually from a second animal species, as well as insertion of disrupted homologous genes. Alternatively, the endogenous *KCNQ2* or *KCNQ3* gene of the animals may be  
10 disrupted by insertion or deletion mutation or other genetic alterations using conventional techniques (Capecchi, 1989; Valancius and Smithies, 1991; Hasty et al., 1991; Shinkai et al., 1992; Mombaerts et al., 1992; Philpott et al., 1992; Snouwaert et al., 1992; Donehower et al., 1992). After test substances have been administered to the animals, the presence of BFNC, rolandic epilepsy or JME must be assessed. If the test substance prevents or suppresses the appearance of BFNC,  
15 rolandic epilepsy or JME, then the test substance is a candidate therapeutic agent for treatment of BFNC, rolandic epilepsy or JME. These animal models provide an extremely important testing vehicle for potential therapeutic products.

The identification of the association between the *KCNQ2* and *KCNQ3* gene mutations and BFNC, rolandic epilepsy and JME permits the early presymptomatic screening of individuals to  
20 identify those at risk for developing BFNC, rolandic epilepsy or JME. To identify such individuals, *KCNQ2* and/or *KCNQ3* alleles are screened for mutations either directly or after cloning the alleles. The alleles are tested for the presence of nucleic acid sequence differences from the normal allele using any suitable technique, including but not limited to, one of the following methods: fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single  
25 stranded conformation analysis (SSCP), linkage analysis, RNase protection assay, allele specific oligonucleotide (ASO), dot blot analysis and PCR-SSCP analysis. Also useful is the recently developed technique of DNA microchip technology. For example, either (1) the nucleotide sequence of both the cloned alleles and normal *KCNQ2* or *KCNQ3* gene or appropriate fragment (coding sequence or genomic sequence) are determined and then compared, or (2) the RNA  
30 transcripts of the *KCNQ2* or *KCNQ3* gene or gene fragment are hybridized to single stranded whole genomic DNA from an individual to be tested, and the resulting heteroduplex is treated with

Ribonuclease A (RNase A) and run on a denaturing gel to detect the location of any mismatches. Two of these methods can be carried out according to the following procedures.

The alleles of the *KCNQ2* or *KCNQ3* gene in an individual to be tested are cloned using conventional techniques. For example, a blood sample is obtained from the individual. The genomic DNA isolated from the cells in this sample is partially digested to an average fragment size of approximately 20 kb. Fragments in the range from 18-21 kb are isolated. The resulting fragments are ligated into an appropriate vector. The sequences of the clones are then determined and compared to the normal *KCNQ2* or *KCNQ3* gene.

Alternatively, polymerase chain reactions (PCRs) are performed with primer pairs for the 5' region or the exons of the *KCNQ2* or *KCNQ3* gene. PCRs can also be performed with primer pairs based on any sequence of the normal *KCNQ2* or *KCNQ3* gene. For example, primer pairs for one of the introns can be prepared and utilized. Finally, RT-PCR can also be performed on the mRNA. The amplified products are then analyzed by single stranded conformation polymorphisms (SSCP) using conventional techniques to identify any differences and these are then sequenced and compared to the normal gene sequence.

Individuals can be quickly screened for common *KCNQ2* or *KCNQ3* gene variants by amplifying the individual's DNA using suitable primer pairs and analyzing the amplified product, e.g., by dot-blot hybridization using allele-specific oligonucleotide probes.

The second method employs RNase A to assist in the detection of differences between the normal *KCNQ2* or *KCNQ3* gene and defective genes. This comparison is performed in steps using small (~500 bp) restriction fragments of the *KCNQ2* or *KCNQ3* gene as the probe. First, the *KCNQ2* or *KCNQ3* gene is digested with a restriction enzyme(s) that cuts the gene sequence into fragments of approximately 500 bp. These fragments are separated on an electrophoresis gel, purified from the gel and cloned individually, in both orientations, into an SP6 vector (e.g., pSP64 or pSP65). The SP6-based plasmids containing inserts of the *KCNQ2* or *KCNQ3* gene fragments are transcribed *in vitro* using the SP6 transcription system, well known in the art, in the presence of [ $\alpha$ -<sup>32</sup>P]GTP, generating radiolabeled RNA transcripts of both strands of the gene.

Individually, these RNA transcripts are used to form heteroduplexes with the allelic DNA using conventional techniques. Mismatches that occur in the RNA:DNA heteroduplex, owing to sequence differences between the *KCNQ2* or *KCNQ3* fragment and the *KCNQ2* or *KCNQ3* allele subclone from the individual, result in cleavage in the RNA strand when treated with RNase A. Such mismatches can be the result of point mutations or small deletions in the individual's allele.

Cleavage of the RNA strand yields two or more small RNA fragments, which run faster on the denaturing gel than the RNA probe itself.

Any differences which are found, will identify an individual as having a molecular variant of the *KCNQ2* or *KCNQ3* gene and the consequent presence of BFNC, rolandic epilepsy or JME.

5 These variants can take a number of forms. The most severe forms would be frame shift mutations or large deletions which would cause the gene to code for an abnormal protein or one which would significantly alter protein expression. Less severe disruptive mutations would include small in-frame deletions and nonconservative base pair substitutions which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic to an acidic  
10 amino acid or vice versa, from a hydrophobic to hydrophilic amino acid or vice versa, or other mutations which would affect secondary or tertiary protein structure. Silent mutations or those resulting in conservative amino acid substitutions would not generally be expected to disrupt protein function.

Genetic testing will enable practitioners to identify individuals at risk for BFNC, rolandic  
15 epilepsy or JME, at, or even before, birth. Presymptomatic diagnosis of these epilepsies will enable prevention of these disorders. Finally, this invention changes our understanding of the cause and treatment of BFNC, rolandic epilepsy and JME. It is possible, for example, that potassium channel opening agents will reduce the risk of seizures in patients with *KCNQ2* or *KCNQ3* mutations.

## 20 Pharmaceutical Compositions and Routes of Administration

The *KCNQ2* and *KCNQ3* polypeptides, antibodies, peptides and nucleic acids of the present invention can be formulated in pharmaceutical compositions, which are prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA). The composition may  
25 contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may comprise, in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for  
30 administration, e.g., intravenous, oral, intrathecal, epineural or parenteral.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing

the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, WO 96/11698.

For parenteral administration, the compound may be dissolved in a pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

The active agent is preferably administered in a therapeutically effective amount. The actual amount administered, and the rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc., is within the responsibility of general practitioners or specialists, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in Remington's Pharmaceutical Sciences.

Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

Instead of administering these agents directly, they could be produced in the target cell, e.g. in a viral vector such as described above or in a cell based delivery system such as described in U.S. Patent No. 5,550,050 and published PCT application Nos. WO 92/19195, WO 94/25503, WO

95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635, designed for implantation in a patient. The vector could be targeted to the specific cells to be treated, or it could contain regulatory elements which are more tissue specific to the target cells. The cell based delivery system is designed to be implanted in a patient's body at the desired target site and contains a coding sequence for the active agent. Alternatively, the agent could be administered in a precursor form for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. See for example, EP 425,731A and WO 90/07936.

The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

#### EXAMPLE 1 Southern Blot Analysis

Five micrograms of genomic DNA were cut with *Taq*I and transferred to a nylon membrane. Filters were hybridized overnight at 65°C in PEG hyb (7% PEG, 10% SDA, 50 mM sodium phosphate and 200 µg/ml total human DNA) with the D20S24 plasmid probe labeled by random priming (Stratagene). Filters were washed at 2 x SSC, 0.1% SDS twice at room temperature followed by one wash in 0.5 x SSC, 0.1% SDS at 65°C.

#### EXAMPLE 2 Fluorescence in situ Hybridization

Chromosomes from transformed lymphocytes were prepared using a 30 minute ethidium bromide treatment followed by 3 hours in colcemid. Cells were then pelleted and resuspended in hypotonic solution (0.75 M KCl) for 20 minutes followed by the addition of four to five drops of fresh fixative (3:1 methanol:acetic acid). Cells were again pelleted, vortexed then carefully resuspended in fixative. After three washes in fixative, metaphases were stored at 4°C. Four hundred ng probe was labeled with biotin and hybridized to slides of metaphase spreads using standard hybridization procedures. Probes were then fluorescently tagged with avidin-FITC (Vector) and the signal intensified using biotin-labeled anti-avidin followed by avidin-FITC. The chromosomes were then counterstained using DAPI and visualized using a Zeiss Axioplan



Fluorescent microscope equipped with FITC, DAPI and triple band pass filter sets. Images were captured by computer using Applied Imaging (Pittsburgh, PA) software Probevision and photographs printed on a Kodak XL 7700 color image printer.

5

### EXAMPLE 3

#### Localization of *KCNO2*

Linkage analysis in a large kindred demonstrated that a gene responsible for BFNC maps to chromosome 20q13.3 close to the markers D20S20 and D20S19 (Leppert et al., 1989). Following the initial report, two centers confirmed linkage of BFNC to the same two genetic markers on  
10 chromosome 20, termed the EBN1 (epilepsy benign neonatal type 1) locus (Ryan et al., 1991; Malafosse et al., 1992). A more distal marker, D20S24, shows complete co-segregation with the BFNC phenotype in chromosome 20 linked families. Finding a distal flanking marker for the BFNC locus has not been successful probably because of its proximity to the telomere. This telomeric region is characterized by a high recombination rate between markers when compared to the  
15 physical distance (Steinlein et al., 1992). In fact, Steinlein et al. have demonstrated that the three markers D20S19, D20S20 and D20S24 are contained on the same 450 Mb Mlu I restriction fragment (Steinlein et al., 1992).

A second chromosomal locus, EBN2, has also been identified for BFNC. Lewis et al. (1993) demonstrated linkage to markers on chromosome 8q24 in a single Hispanic family affected with  
20 BFNC. Evidence for this second locus was also reported in a Caucasian pedigree (Steinlein et al., 1995). All of the families in the present study show linkage to chromosome 20q markers with LOD scores of greater than 3.0 or have probands with clinical manifestations consistent with BFNC (Leppert et al., 1993). To find the gene responsible for BFNC, we narrowed the BFNC region with a sub-microscopic deletion in a single family, identified candidate cDNAs in this deletion, and then  
25 searched for mutations in other BFNC families.

Evidence for a small deletion came first from a genotypic observation with a three allele, RFLP marker, D20S24. Analysis of one family, kindred 1547, revealed that a null allele occurred exclusively in those individuals with BFNC and in two individuals previously shown to be non-penetrant with the VNTR markers D20S20 and D20S19 (Figure 1). The existence of a deletion co-  
30 segregating with the BFNC phenotype in this family was confirmed by fluorescence in situ hybridization (FISH) in cell lines of kindred 1547 individuals using as probes, the D20S24 plasmid and two genomic P1 clones containing this marker.

To confirm the presence of a deletion, two overlapping genomic P1 clones, P1-KO9-6b and P1-KO9-7, each of approximately 80 kb in size and each of which contains the D20S24 marker, were obtained and these were hybridized to cell lines of kindred 1547 BFNC affected individuals. When metaphase spread chromosomes are hybridized with P1-KO9-7 and P1-KO9-6b, both chromosome 20 homologs give signals on two sister chromatids. However when the 12 kb probe D20S24 is hybridized only signal from the one chromosome homolog is observed in 75% of metaphase spreads examined. The remaining minority of cells showed no hybridization for the 12 kb D20S24 probe (Figure 2). The plasmid containing the D20S24 marker was a kind gift from J. Weissenbach.

While the 12 kb D20S24 probe was deleted on one chromosome in affected individuals, the overlapping P1 clones of 80 kb in size, and which together span approximately 130 kb, showed a positive FISH signal indicating that the deletion is smaller than 130 kb (Figure 2).

#### EXAMPLE 4

##### Isolation and Characterization of *KCNQ2* Clones

Using the same probes as in Example 3, cDNAs in the region of the deletion were identified by screening a fetal brain cDNA library. Three of the cDNAs isolated showed significant homology to *KCNQ1*, the chromosome 11 potassium channel gene responsible for the Long QT syndrome and the Jervell and Lange-Nielsen cardioauditory syndrome (Wang et al., 1996; Altschul et al., 1990; Neyroud et al., 1997).

A fetal brain cDNA library (Stratagene) ( $10^6$  clones) was probed with inserts from P1-KO9-6b and P1-KO9-7 and the plasmid D20S24. Hybridizations were performed in 5 x SSC, 10 x Denhardt's, 0.1 M sodium phosphate (pH 6.7), 100 µg/mL salmon sperm DNA, 0.1% SDS and 50% formamide. Blots were washed in 2 x SSC, 0.1% SDS twice at room temperature followed by one wash in 0.5 x SSC, 0.1% SDS at 42°C.

A single cDNA isolated with D20S24, cIPK, showed 75% homology to amino acids 511-562 of *KCNQ1*; a second probing of the fetal brain cDNA library using the probe P1-KO9-6b resulted in the isolation of two additional cDNAs, c6b-6 and c6b-12, which showed significant homology with *KCNQ1* amino acids 398-406 and 354-378, respectively (Altschul et al., 1990; Wang et al., 1996; Neyroud et al., 1997).

Additional sequence encoding this BFNC gene, named *KVEBN1* (now *KCNQ2*) after the OMIM locus name, was obtained from RACE experiments using adaptor-ligated double-stranded

cDNA from fetal and adult brain tissue and from other cDNA clones isolated from a temporal cortex cDNA library.

To identify the full length gene, 5' and 3' RACE were performed on adaptor-ligated fetal and adult brain cDNA (Clontech) using primers within c6b-6 and cIPK and screening a temporal cortex  
5 cDNA library (Stratagene) with sequence flanking cIPK. Unprocessed cDNAs were repeatedly isolated from cDNA libraries and RACE experiments. The longest transcript isolated from brain was 1455 nucleotides long and was obtained using 5' RACE and extended from the S1 domain (amino acid 100) to the 3' conserved C-terminal domain (amino acid 585).

Composite clones encoding 872 amino acids of the KCNQ2 gene have been isolated (Figure  
10 3). The cDNA sequence for *KCNQ2* is shown as SEQ ID NO:1 and the amino acid sequence for KCNQ2 is shown as SEQ ID NO:2. The putative initiator methionine lies within a region similar to the Kozak consensus sequence (Kozak, 1987). KCNQ2 encodes a highly conserved six transmembrane motif as well as a pore region that are the hallmarks of a K<sup>+</sup> ion channel gene. The S2, S3 and S4 transmembrane regions also contain charged amino acids that are found in all  
15 members of the K<sup>+</sup> channel subfamilies, including *Shaker*, *Shab*, *Shaw* and *Shal*. A search of Genbank with KCNQ2 sequence shows identical nucleotide sequence to HNSPC (Accession # D82346), a 393 amino acid putative potassium channel cDNA isolated from a human neuroblastoma cell line (Yokoyama et al., 1996). However, the last 21 amino acids of HNSPC including a stop codon are encoded by a sequence that in *KCNQ2* is intronic. A search of the human expressed  
20 sequence tag database (dbest) shows seven different clones encoding portions of KCNQ2. Wei et al. have identified a gene from *C. elegans*, *nKQT1*, that appears to be a homolog of *KCNQ2* (Wei et al., 1996). This group also described the human EST homolog of *nKQT1*, *hKQT2*, which is a partial clone of *KCNQ2* (Wei et al., 1996). In addition to the six transmembrane domains and the pore, a small region 5' of transmembrane domain S1 is also conserved between KCNQ2, KCNQ3,  
25 KCNQ1 and nKQT1. Unlike other K<sup>+</sup> channel subfamilies, the C-terminal domain appears to contain highly conserved residues as shown in Figure 3 for KCNQ2, KCNQ3, nKQT1 and KCNQ1. The poly A tail for *KCNQ2* has not been identified to date.

### EXAMPLE 5

30

#### Northern Blot Analysis

The *KCNQ2* cDNA hybridizes to transcripts approximately 1.5, 3.8 and 9.5 kb in size on Northern blots made from brain. Multiple Tissue Northern (Clontech) of fetal and adult brain were

probed with a RACE product containing transmembrane domains S1 through S6 of KCNQ2. The 1.5 and 9.5 kb transcripts appear to be expressed in both adult and fetal brain. The 3.8 kb transcript is expressed in select areas from adult brain, particularly in the temporal lobe and the putamen.

5

### EXAMPLE 6

#### Mutational Analysis of KCNQ2

Mutational analysis of *KCNQ2* was performed on one affected individual from each of our 12 BFNC families. Coding regions from S1 to S6 and conserved regions in the 3' end of *KCNQ2* were amplified by PCR using primers within introns and analyzed by SSCP (Novex) using 20% TBE gels run at 4°C. The exon-intron boundaries were identified by sequencing products obtained by exon-exon PCR on genomic P1 clones or directly from RACE products which contained unprocessed transcripts. PCR products showing variants seen on SSCP were either cloned and sequenced or reamplified with M13 reverse and M13 universal-tailed primers and sequenced directly on an ABI 373 or 377 using dye-primer chemistry.

15 In addition to the substantial deletion in kindred 1547, mutations were identified in five other BFNC families. Mutational analysis was carried out by first screening probands for SSCP variants and then sequencing each individual's DNA to determine the basis for the molecular variation. Mutations identified include two missense mutations, two frameshift mutations and one splice site mutation (Table 2). Later analyses resulted in the finding of four more BFNC families with 20 mutations in *KCNQ2*. These include two nonsense mutations (families K1525 and K4443), an insertion resulting in a frameshift which results in readthrough beyond the normal stop codon (K3963), and a missense mutation (K4516). These latter 4 mutations are listed in Table 2.

The splice site variant occurs in an intron which occurs between two exons encoding amino acid residue 544. The first exon includes the TG at the start of codon 544 and the following exon 25 includes the final T of codon 544. The sequence at the 3' end of the intron (shown in lower case letters) and continuing into the exon region (shown in upper case letters) encoding the end of codon 544 and codons 545-546 is: 5'-tgcagTGTCATG-3' (SEQ ID NO:5). The "g" at position 5 of SEQ ID NO:5 is mutated to an "a" in kindred K3933.

30 None of the mutations seen in the first six families identified was seen in SSCP analysis of our panel of 70 unrelated, unaffected individuals. Furthermore, mutations were shown to segregate completely with affection status in all of the BFNC families where mutations were identified. In the case of the splice site mutation in kindred 3933 only the proband was sampled. An example

Table 2Mutations in the *KCNQ2* Gene in BFNC Families

	Mutation at Amino Acid	Region	Kindred	Controls	Nucleotide Change
5	large deletion	not available	K1547	70	not available
	frameshift at 283	pore	K1504	70	insert GT between nucleotides 975 and 976 of SEQ ID NO:1
	Y284C	pore	K3904	70	A→G at base 978 of SEQ ID NO:1
	A306T	S6	K1705	70	G→A at base 1043 of SEQ ID NO:1
	Q323Stop	C-terminal	K4443	--	C→T at base 1094 of SEQ ID NO:1
10	R333Q	C-terminal	K4516	--	G→A at base 1125 of SEQ ID NO:1
	R448Stop	C-terminal	K1525	--	C→T at base 1469 of SEQ ID NO:1
	frameshift at 522	C-terminal	K3369	70	delete bases 1691 through 1703 of SEQ ID NO:1
	splice site variant	C-terminal	K3933	70	g→a at 3' end of intron which occurs between bases 1758 and 1759 of SEQ ID NO:1
	frameshift at 867	C-terminal	K3963	70	insert GGGCC after base 2736 of SEQ ID NO:1

of this segregation is shown in Figure 4 for the two base-pair insertion identified in kindred 1504; all 11 affected members of the kindred have the SSCP variant and all seven unaffected individuals have wild type SSCP bands.

Of the four families (K1525, K3963, K4443 and K4516) which have been more recently found to have *KCNQ2* mutations, three (K1525, K4443 and K4516) were found through direct sequencing and the mutation co-segregated in the family when other affected members were available for study. The mutation in K3963 was found via SSCP screening and this mutation was not detected in a panel of 70 normal, i.e., non BFNC, individuals. This mutation was found to co-segregate with affected individuals in family K3963. The wild-type gene includes two sets of GGGCC at bases 2727-2736 of SEQ ID NO:1. The sequence found in K3963 is three sets of GGGCC as a result of an insertion of GGGCC into this region. This results in the gene encoding the first 870 amino acids of the wild-type followed by an additional 60 amino acids of new sequence (amino acid residues 871 and 872 of the wild-type being replaced by the first 2 of the 60 additional amino acid residues). The gene including the 5 base insertion is shown as SEQ ID NO:95 and the protein encoded by this mutated gene is shown as SEQ ID NO:96.

Family K4443 has 6 BFNC affected individuals and two of these individuals have in addition seizures later in childhood that are classified as benign epilepsy with centrotemporal spikes (BERS), or rolandic epilepsy. The DNA of two affected individuals in this family was examined. The Q323Stop mutation is found in one of the affected individuals that expresses BFNC only and in one individual which has both BFNC and BERS or rolandic epilepsy, which developed later in childhood after the newborn seizures. This finding directly implicates the *KCNQ2* gene on chromosome 20 in causing rolandic epilepsy. Rolandic epilepsy, or BERS, is a common childhood epilepsy and may account for 25% of all school age epilepsy. This is a genetic disorder that inherits as an autosomal dominant with reduced penetrance. It is possible that several genes may cause the rolandic phenotype, but this finding strongly suggests that at least some of the rolandic epilepsies will be caused by defects in *KCNQ2*, a potentially important finding.

Two neutral polymorphisms were identified in the *KCNQ2* gene. One polymorphism is in codon 304 (TTC to TTT) in the S6 transmembrane domain and was seen in 10 of 71 controls who were each heterozygous (allelic frequency of 7.0%). The second polymorphism is in codon 573 (GCC to GCT) in the 3' region and was observed in 1 of 87 controls individuals as a heterozygote (allelic frequency of 0.57%).

It is predicted that the splice site mutation in the conserved 3' region of *KCNQ2* and the two frameshift mutations, one in the pore region and one before the highly conserved 3' region, lead to altered protein products. In the case of the 283insGT pore mutation a predicted stop codon is found 36 amino acids downstream and in the case of the 522del13 3' mutation a predicted stop codon is found two amino acids downstream. Also, the two bp insertion mutation, 283insGT, would lead to a completely altered S6 transmembrane domain. While the breakpoints of the kindred 1547 deletion have not been determined, it is known that the 12 kb plasmid which includes the RFLP marker locus, D20S24, contains 80 codons (residues 509 to 588 of *KCNQ2*) of sequence from the highly conserved 3' region of the *KCNQ2* gene, indicating that at least this portion of the gene is deleted in kindred 1547 affected individuals. The two missense mutations in families K3904 and K1705 change amino acid residues in key functional domains, the pore and S6 domain.

Ten unique mutations have been identified in *KCNQ2* to date. The mutation defined by a 13 base pair deletion at amino acid 522 in kindred 3369 is of interest in that there is a greater variation in the reported clinical ages of onset within this family when compared to typical BFNC families. In kindred 3369, three individuals had onset of seizures within the first 2 weeks of life, while three individuals had initial onset of seizures at 3, 4, and 5 months of age.

The mutation in the BFNC kindred 1705 is an alanine to threonine substitution in the S6 transmembrane segment. This alanine residue is conserved in all members of the *Shaker*, *Shab*, *Shaw* and *Shal* subfamilies of potassium channels identified to date (Lopez et al., 1994; Nakamura et al., 1997; Tytgat, 1994). The *KCNQ1* gene, which the *KCNQ2* ion channel gene is most closely related to, also contains an alanine in this position. In six unrelated LQT1 families, the disease-causing mutation occurs at this same position where the alanine is changed to a valine (Wang et al., 1996; Russell et al., 1996). This S6 transmembrane domain has been shown to be involved in K<sup>+</sup> ion permeation in the *Shaker* subtype (Lopez et al., 1994) and may serve a similar function in *KCNQ2*. The C-terminal region appears to be important for gene function because a 13 bp deletion, a splice site mutation, a missense mutation, a nonsense mutation, and an insertion all produce an epileptic phenotype in separate BFNC families (see Table 2 and Figure 3). Interestingly, this same region is known to have a deletion-insertion mutation in *KCNQ1* in individuals with the Jervell and Lange-Nielsen recessive form of LQT and associated deafness (Neyroud et al., 1997). Disease-causing mutations in the C-terminal region further argue for a functional protein encoded by the *KCNQ2* gene rather than the shorter HNSPC clone.

The pore region of K<sup>+</sup> ion channels belonging to the same structural class have been characterized extensively by mutational analysis. The two base-pair insertion observed in kindred 1504 occurs immediately after the universally conserved GYG motif. An insertion here not only alters the length of the pore that is believed to be crucial for function (Nakamura et al., 1997; Tytgat, 5 1994) but also modifies the signature sequence of the pore and produces a truncated protein.

In infants of families that have been linked to the chromosome 20 form of BFNC, EEG recordings show initial suppression of activity throughout the brain followed by generalized discharges of spikes and slow waves (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975). It is therefore not surprising to find that the *KCNQ2* gene is expressed in multiple brain areas in 10 adults. Cortical regions as well as sub-cortical areas, such as the thalamus and caudate nucleus, contain multiple size transcripts of *KCNQ2* (data not shown). It is possible that this expression pattern is also the same in the newborn infant.

The close homology (60% identity and 70% similarity of amino acids) of *KCNQ2* to *KCNQ1* and to the *C. elegans nKQT1* gene and the reduced homology of these channels to the *Shaker*, *Shab*, 15 *Shaw* and *Shal* subfamilies imply that they belong to a unique family of K<sup>+</sup> ion channels, called KQT-like (Wei et al., 1996). A new K<sup>+</sup> ion channel now known to be expressed in the brain raises the question of whether additional, undiscovered members of this gene family may be responsible for other forms of idiopathic, generalized epilepsies with tonic-clonic convulsions. A similar idiopathic seizure disorder seen early in development is Benign Familial Infantile Convulsions 20 (BFIC). In BFIC the seizures begin at four to eight months of age and remit after several years. BFIC maps to chromosome 19q in five Italian families (Guipponi et al., 1997). It is reasonable to hypothesize that BFIC is also caused by mutations in as yet unidentified members of the KQT-like family of K<sup>+</sup> ion channels or by minK-like proteins.

25

## EXAMPLE 7

Somatic Cell Hybrid Panel Genotyping

Exploiting the putative conservation of intron-exon boundaries between *KCNQ2* and *KCNQ3* in the highly homologous transmembrane domains, a primer pair was designed from the available EST sequences (primer A: 5'-TTCCTGATTGTCCTGGGGTGCT-3' (SEQ ID NO:8), 30 primer B: 5'-TTCATCTTTGGAGCCGAGTTTGC-3' (SEQ ID NO:9)) to cross an intron. The amplified fragment contains an intron in human (1.8 kb) as well as in rodent (800 bp) genomic DNA. This primer was used to amplify the Coriell panel. The reactions were performed in a 25 µL



volume using 50 ng of template DNA and 1 unit of Taq DNA polymerase (Perkin Elmer), 10 pmol of each primer, 3 nmol of each deoxyribonucleotide in a 1.5 mM MgCl<sub>2</sub> buffer. Cycling conditions were 94°C for 4 minutes, then 30 cycles of: denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds and elongation at 72°C for 1.5 minutes, followed by a final elongation at 72°C for 10 minutes. The PCR products were electrophoresed in a 1.5% agarose gel.

#### EXAMPLE 8

##### Chromosome 8 Radiation Hybrids Panel

An HSA8 radiation hybrid panel (Lewis et al., 1995) was genotyped with specific human intronic primers (primer D: 5'-TCCATGTGGTACTCCATGTCTGAA-3' (SEQ ID NO:10), primer E: 5'-GCACGTCACATTGGGGATGTCAT-3' (SEQ ID NO:11)). The length of the PCR product is 190 bp. The reactions were performed in a 25 µL volume using 100 ng of template DNA and 1 unit of Taq polymerase (Perkin Elmer), 10 pmol of each primer, 3 nmol of each deoxyribonucleotide in a 1.5 mM MgCl<sub>2</sub> buffer. Cycling conditions were 94°C for 4 minutes, then 30 cycles of: denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds and elongation at 72°C for 30 seconds, followed by a final elongation at 72°C for 10 minutes. The PCR products were electrophoresed in a 2% agarose gel. The genotyping data was analyzed by the RHMAP V2.01 program (Boehnke et al., 1991).

#### EXAMPLE 9

##### Full Length cDNA

To identify the full length *KCNQ3* cDNA, 5' and 3' RACE were performed on adaptor-ligated fetal and adult brain cDNA (Clontech) using primers from the available EST sequences. The primers used for RACE experiments are given in Table 3. PCR products were subcloned (T/A cloning® Kit, Invitrogen) and both strands were sequenced on an ABI 377 instrument.

#### EXAMPLE 10

##### Genomic Organization/Intron-Exon Boundaries

A BAC genomic library was screened by PCR (as described for the Coriell panel) and three overlapping genomic clones were isolated. The intron/exon boundaries were identified by cloning (T/A cloning® Kit, Invitrogen) and sequencing (ABI 377) products obtained by exon-exon PCR on genomic human DNA and/or on BAC genomic clones containing the *KCNQ3* gene.

Table 3  
RACE Primers

5' RACE

5	KV1b: 5'-TGTGTTTTTGGCGTGGAGGGAGGTC-3'	(SEQ ID NO:12)
	KV2b: 5'-CAGTAACAGAAGCCAGTCTCC-3'	(SEQ ID NO:13)
	KV3b: 5'-GCAAACTCGGCTCCAAAGATGAA-3'	(SEQ ID NO:14)
	<u>KV4b: 5'-CACCAACGCGTGGTAAAGCAGC-3'</u>	<u>(SEQ ID NO:15)</u>

10

3' RACE

	KV1a: 5'-TTCCTGATTGTCCTGGGGTGCT-3'	(SEQ ID NO:16)
	<u>KV2a: 5'-AGTATCTGCCGGGCATCTCGACA-3'</u>	<u>(SEQ ID NO:17)</u>

## EXAMPLE 11

SSCP Analysis and Characterization of Mutant and Polymorphic Alleles

Sixty percent of the coding region of *KCNQ3* was amplified by PCR using primers within introns when available and analyzed by SSCP (Novex) using 20% TBE gels run at 4°C as described in Novex ThermoFlow™ protocols (Novex, San Diego, CA). The PCR products presenting an SSCP polymorphism were cloned (T/A cloning® Kit, Invitrogen), nine clones were sequenced on an ABI 373 or 377 using dye-primer chemistry and analyzed with the Sequencer™ 3.0 program.

## EXAMPLE 12

Characterization of the KCNQ3 Gene

The KQT-like family is a recently characterized family of voltage-gated potassium channels (Wei et al., 1996). Until now, only *KCNQ2* (described in this disclosure) which is the gene mutated in the chromosome 20 BFNC disorder and *KCNQ1*, which is the chromosome 11 gene responsible for Long QT syndrome and the Jervell and Lange-Nielsen cardioauditory syndrome (Neyroud et al., 1997), were known to belong to this family. In order to identify new members of that family, possibly involved in other types of IGEs, a tBLASTx (Altschul et al., 1990) search was started with the *KCNQ2* full length cDNA against the Expressed Sequence Tags (ESTs) database. Five human EST clones were identified that presented significant homologies with *KCNQ2* (clone ID: 1-362079, 2-222324, 3-363215, 4-38822, 5-45636; Hillier et al., unpublished data). Interestingly, these clones come from two different cDNA libraries: retina (1-3) and infant brain (4-5) (Soares et al., 1994) and can be organized in two nonoverlapping contigs (1-3) and (4-5). It is demonstrated here that the two contigs belong to the same gene, *KCNQ3*.

The first step in the characterization of the new gene was genomic localization of the ESTs. Using a commercial somatic cell hybrid panel (Coriell panel (Drwinga et al., 1993)), *KCNQ3* was mapped on HSA8. In order to refine that assignment, a panel of 97 radiation hybrids previously constructed for determining the linear order and intermarker distance of chromosome 8 loci (Lewis et al., 1995) was genotyped. Specific human intronic primers were used and each RH was scored by PCR for the presence or absence of the locus. The data were analyzed using RHMAP V2.01 against results collected for other chromosome 8 markers. The retention frequency for *KCNQ3* in the RH panel was 11.7%. Tight linkage of *KCNQ3* locus was observed with markers previously mapped to chromosome band 8q24. The tightest linkage was seen with marker D8S558 (LOD 13.87,  $\theta$  of 0.047  $R_{5000}$ ). The resulting RH map is shown in Figure 5. The position of the *KCNQ3*

locus is localized to the interval defined by the markers previously linked to a chromosome 8 BFNC family (Lewis et al., 1993), making *KCNQ3* a very strong positional candidate for the chromosome 8 BFNC locus. A second Caucasian family also demonstrates suggestive linkage to the same markers (Steinlein et al., 1995).

5 A partial cDNA sequence was obtained by a series of rapid amplification of cDNA ends (RACE) experiments. 5' and 3' RACE were performed by amplifying adult and fetal brain Marathon-Ready cDNAs (Clontech) using primers derived from the two EST contigs previously identified. The primer pairs are shown in Table 3. This was used to purify a mouse genomic homolog of *KCNQ3*. After determining the mouse sequence including intron/exon junctions,  
10 primers based upon the mouse sequence were used to clone the remainder of the human cDNA for *KCNQ3*. The primers used to amplify the 5' end of the human gene were CGCGGATCATGGCATTGGAGTTC (SEQ ID NO:93) and AAGCCCCAGAGACTTCTCAGCTC (SEQ ID NO:94). The complete *KCNQ3* cDNA sequence (SEQ ID NO:6) encodes an 872 amino acid protein (SEQ ID NO:7) with six putative transmembrane domains, a pore region, a stop codon,  
15 and the 3' untranslated region containing the poly A<sup>+</sup> tail. This protein presents 58% similarity and 46% identity (calculated using BLAST) in the region from amino acid 101 to the stop codon with *KCNQ2* and is also highly conserved with *KCNQ1* (Yang et al., 1997) as well as with the *C. elegans* homologue nKQT1 (Wei et al., 1996). A comparison of sequences is shown in Figure 3. The two EST contigs are identical to amino acids 86-265 and 477-575 of *KCNQ3*, respectively (see  
20 Figure 3).

To test whether or not *KCNQ3* is the gene responsible for the chromosome 8 BFNC phenotype, mutations were looked for in one affected individual of a phenotypically well characterized three-generation Mexican-American BFNC family (Ryan et al., 1991) (see Figure 6). That family has been mapped by multipoint linkage analysis on chromosome 8q24 (Z=4.43) within  
25 the interval spanned by markers D8S198 (proximal to D8S284) and D8S274 (distal to D8S256) (see Figure 5) (Lewis et al., 1993; Dib et al., 1996). It is here shown that this chromosomal region contains the *KCNQ3* locus. So far, using intronic primers, 60% of the coding region of *KCNQ3*, containing the six transmembrane domains as well as the pore region, has been screened by a cold SSCP method. One SSCP variant was identified in a PCR fragment of 187 bp containing the  
30 transmembrane domain S5 and half of the pore. The primers used to prepare this fragment are: Ret.6a 5'-CATCACGGCCTGGTACATCGGTT-3' (SEQ ID NO:18) (corresponding to nucleotides 801-823 of SEQ ID NO:6) and Hebn2.3b 5'-AATCTCACAGAATTGGCCTCCAAG-3' (SEQ ID

NO:19). The Ret.6a primer is from coding region and the Hebn2.3b primer is from intronic region. This SSCP variant is in perfect cosegregation with the BFNC phenotype and it is also present in a single non-penetrant individual carrying the disease-marker haplotype (Figure 6). Furthermore, this SSCP variant is absent from a panel of 72 Caucasian and 60 Mexican-American (264 chromosomes) unrelated individuals used as the control group. To characterize the nucleotide change of this variant, the PCR product of one affected individual was cloned and nine clones were sequenced on both strands. Four clones contained the wild-type allele and five the mutated allele. The mutation is a single missense mutation Gly (GGC) to Val (GTC) in position 310 of the highly conserved pore region (the mutation occurring at base 947 of SEQ ID NO:6). In addition, a silent polymorphism (frequency of 0.4%) was found in one Mexican-American control in the transmembrane region S5 at L278 (CTT – CTC) (the polymorphism is at base 852 of SEQ ID NO:6). Four other polymorphisms in KCNQ3 have been seen. These are at N220 (AAC or AAT), Gly244 (GGT or GGC), L357 (CTG or CTC) and I860 (ATT or ATC). These polymorphisms are at base numbers 678, 750, 1089 and 2598 of SEQ ID NO:6, respectively.

In addition, some individual probands with juvenile myoclonic epilepsy were screened with SSCP. JME is an inherited childhood seizure disorder. KCNQ3 was mutated in one individual who was tested. The mutation was found in an alternatively spliced exon that lies in an intron which splits codon 412. This alternatively spliced exon was found in adult brain after RACE experiments. This exon is SEQ ID NO:92. The exon was seen in an adult brain cDNA clone obtained from Clontech. This exon is 130 nucleotides long which is not a multiple of 3. Therefore the presence of this exon results not only in the addition of extra amino acid sequence but causes a frameshift (1 extra base) which results in a stop codon within the normal coding region of the gene. The mutation found in the JME proband is a 1 base pair deletion in the alternatively spliced exon (the loss of the G at base 118 of SEQ ID NO:92) that results in the frameshift from the alternative exon going back into frame resulting in a KCNQ3 with an additional 43 amino acid residues between amino acid residues 412 and 413 of the wild-type, and thus alters the protein in the brain cells of the JME proband. The patient with this deletion has a mother who has epilepsy, however this particular mutation is from the father, not from the mother. JME is a common, inherited childhood epilepsy and most likely is caused by defects not only in KCNQ3 but also in other genes.

This finding brings to three the number of human members of the KQT-like family, two of which are expressed in brain and one in heart. Defects in all three K<sup>+</sup> channel genes cause human diseases associated with altered regulation of excitability. Taking all these findings together, there

is strong evidence that *KCNQ2* and *KCNQ3*, as well as undiscovered genes of the same family or genes belonging to the same pathway, are involved in IGEs. Screening these KQT-like K<sup>+</sup> channel genes as well as other K<sup>+</sup> channel genes belonging to different families (Wei et al., 1996) for mutations in individuals with common types of IGEs will be a powerful alternative for identifying disease-causing genes. This is especially true given the difficult and controversial tentative linkages described in IGE disease pedigrees (Leppert et al., 1993).

### EXAMPLE 13

#### Generation of Polyclonal Antibody against KCNQ2 or KCNQ3

10 Segments of *KCNQ2* or *KCNQ3* coding sequence are expressed as fusion protein in *E. coli*. The overexpressed protein is purified by gel elution and used to immunize rabbits and mice using a procedure similar to the one described by Harlow and Lane, 1988. This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

Briefly, a stretch of *KCNQ2* or *KCNQ3* coding sequence is cloned as a fusion protein in  
15 plasmid PET5A (Novagen, Inc., Madison, WI). After induction with IPTG, the overexpression of a fusion protein with the expected molecular weight is verified by SDS/PAGE. Fusion protein is purified from the gel by electroelution. Identification of the protein as the *KCNQ2* or *KCNQ3* fusion product is verified by protein sequencing at the N-terminus. Next, the purified protein is used as immunogen in rabbits. Rabbits are immunized with 100 µg of the protein in complete Freund's  
20 adjuvant and boosted twice in 3 week intervals, first with 100 µg of immunogen in incomplete Freund's adjuvant followed by 100 µg of immunogen in PBS. Antibody containing serum is collected two weeks thereafter.

This procedure is repeated to generate antibodies against the mutant forms of the *KCNQ2* or *KCNQ3* gene product. These antibodies, in conjunction with antibodies to wild type *KCNQ2* or  
25 *KCNQ3*, are used to detect the presence and the relative level of the mutant forms in various tissues and biological fluids.

### EXAMPLE 14

#### Generation of Monoclonal Antibodies Specific for KCNQ2 or KCNQ3

30 Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising intact *KCNQ2*, intact *KCNQ3*, *KCNQ2* peptides or

KCNQ3 peptides (wild type or mutant) conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known.

The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100  $\mu$ g of immunogen and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

Spleens are removed from immune mice and a single cell suspension is prepared (see Harlow and Lane, 1988). Cell fusions are performed essentially as described by Kohler and Milstein, 1975. Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) are fused with immune spleen cells using polyethylene glycol as described by Harlow and Lane, 1988. Cells are plated at a density of  $2 \times 10^5$  cells/well in 96 well tissue culture plates. Individual wells are examined for growth and the supernatants of wells with growth are tested for the presence of KCNQ2 or KCNQ3 specific antibodies by ELISA or RIA using wild type or mutant KCNQ2 or KCNQ3 target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibody for characterization and assay development.

## EXAMPLE 15

### Sandwich Assay for KCNQ2 or KCNQ3

Monoclonal antibody is attached to a solid surface such as a plate, tube, bead or particle. Preferably, the antibody is attached to the well surface of a 96-well ELISA plate. 100  $\mu$ L sample (e.g., serum, urine, tissue cytosol) containing the KCNQ2 or KCNQ3 peptide/protein (wild-type or mutants) is added to the solid phase antibody. The sample is incubated for 2 hrs at room temperature. Next the sample fluid is decanted, and the solid phase is washed with buffer to remove unbound material. 100  $\mu$ L of a second monoclonal antibody (to a different determinant on the KCNQ2 or KCNQ3 peptide/protein) is added to the solid phase. This antibody is labeled with a detector molecule (e.g.,  $^{125}$ I, enzyme, fluorophore, or a chromophore) and the solid phase with the second antibody is incubated for two hrs at room temperature. The second antibody is decanted and the solid phase is washed with buffer to remove unbound material.

The amount of bound label, which is proportional to the amount of KCNQ2 or KCNQ3 peptide/protein present in the sample, is quantified. Separate assays are performed using monoclonal antibodies which are specific for the wild-type KCNQ2 or KCNQ3 as well as monoclonal antibodies specific for each of the mutations identified in KCNQ2 or KCNQ3.

5

#### EXAMPLE 16

##### Assay to Screen Drugs Affecting the KCNQ2 or KCNQ3 K<sup>+</sup> Channel

With the knowledge that KCNQ2 and KCNQ3 each forms a potassium channel, it is now possible to devise an assay to screen for drugs which will have an effect on one or both of these  
10 channels. The gene is transfected into oocytes or mammalian cells and expressed as described above. When the gene used for transfection contains a mutation which causes BFNC, rolandic epilepsy or JME, a change in the induced current is seen as compared to transfection with wild-type gene only. A drug candidate is added to the bathing solution of the transfected cells to test the effects of the drug candidates upon the induced current. A drug candidate which alters the induced  
15 current such that it is closer to the current seen with cells cotransfected with wild-type *KCNQ2* or wild-type *KCNQ3* is useful for treating BFNC, rolandic epilepsy or JME.

#### EXAMPLE 17

##### PRIMER PAIRS FOR SCREENING EACH EXON OF KCNQ2 FOR MUTATION

20 The genomic *KCNQ2* has been sequenced in the intron/exon borders and primer pairs useful for amplifying each exon have been developed. These primer pairs are shown in Table 4. For exons 13 and 17 primers within the exons are also utilized. Some exon/intron sequence is shown in Figures 7A-O.

#### EXAMPLE 18

##### INTRON SEQUENCE OF KCNQ3 AND PRIMER PAIRS FOR AMPLIFYING THE EXONS OF KCNQ3

25

Although the complete cDNA for *KCNQ3* has been obtained and sequenced, the complete genomic DNA has not yet been sequenced. However, much of the intron DNA has been sequenced and this sequence information has been utilized to develop primer pairs which are useful for  
30 amplifying each exon. The intron/exon sequence is shown in Figures 8A-O. Some useful primer pairs for amplifying each exon are shown in Table 5 although one of skill in the art can easily develop other primer pairs using the intron sequence shown in Figures 8A-O.



Table 4

Exon	Domain	Primer Sequence (SEQ ID NO:)
1	met + SI	
2	SI + SII	TTCCTCCTGGTTTTCTCCTGCCT (SEQ ID NO:22) AAGACAGACGCCCAGGCAGCT (SEQ ID NO:23)
5 3	SII + SIII	AGGCCTCAAGGTGGCCTCAGCTTT (SEQ ID NO:24) CTGGCCCTGATTCTAGCAATAC (SEQ ID NO:25)
4	SIII + SIV	ACATCATGGTGCTCATCGCCTCC (SEQ ID NO:97) TGTGGGCATAGACCACAGAGCC (SEQ ID NO:26)
5	SV + pore	TGGTCACTGCCTGGTACATCGG (SEQ ID NO:27) ATGGAGCAGGCTCAGCCAGTGAGA (SEQ ID NO:28)
6	pore + SVI	GCAGGCCCTTCGTGTGACTAGA (SEQ ID NO:29) ACCTAGGGAAGTGTGCCAGG (SEQ ID NO:30)
7	SVI	ATGGTCTGACCCTGATGAATTGG (SEQ ID NO:31) GCGGCCTCCACTCCTCAACAA (SEQ ID NO:32)
10 8	C-term	
9	C-term	
10	variable	CCGCCGGGCACCTGCCACCAA (SEQ ID NO:33) GCTTGCACAGCTCCATGGGCAG (SEQ ID NO:34)
11	C-term	GCTGTGCAAGCAGAGGGAGGTG (SEQ ID NO:98) CTGTCCTGGCGTGTCTTCTGTG (SEQ ID NO:99)
12	variable cysteine insertion	CCCAGGACTAACTGTGCTCTCC (SEQ ID NO:35) CCGTGCAGCAGCCGTCACTCC (SEQ ID NO:36)
15 13	C-term	GCAGAGTGACTTCTCTCCCTGTT (SEQ ID NO:37) GTCCCCGAAGCTCCAGCTCTT (SEQ ID NO:38)  AAGATCGTGTCTTCTCCAGCCC (SEQ ID NO:39) GATGGACCAGGAGAGGATGCGG (SEQ ID NO:40)
14	C-term	CCCTCACGGCATGTGTCCTTCC (SEQ ID NO:41) AGCGGGAGGCCCTCCTCACT (SEQ ID NO:42)
15	C-term	GGTCTCTGGCCCAGGGCTCACA (SEQ ID NO:43) CTTGTCCTGCTGGACAGGCA (SEQ ID NO:44)
16	C-term	TTGACGGCAGGCACCACAGCC (SEQ ID NO:45)

Exon	Domain	Primer Sequence (SEQ ID NO:)
17	C-term	CCCAGCCCAGCAGCCCCTTTT (SEQ ID NO:46) AGGTGGAGGGCGGACACTGGA (SEQ ID NO:47)  CTCCACGGGCCAGAGAAGAACTTC (SEQ ID NO:48) GATGGAGATGGACGTGTCGCTGT (SEQ ID NO:49)  TGGAGTTCCTGCGGCAGGAGGACAC (SEQ ID NO:50) GGTGTCTGACTCTCCCTCCGCAA (SEQ ID NO:51)  GTGGCGCCTTGTGCCAAAGTCA (SEQ ID NO:52) ACCTCGGAGGCACCGTGCTGA (SEQ ID NO:53)

Table 5

Pair	Sequence 5'→3' (SEQ ID NO:)	Size	Temp	Part of the gene
1	GCGACGTGGAGCAAGTACCTTG (54) CACCAACGCGTGGTAAAGCAGC (55)	245	62	before S1
2	ATGACTCAAAGGTTTCCTTAGTCCA (56) GAAGCCCAACCAGAAGCATTAC (57)	174	62	S1 to beginning of S2
3	TCAGTGCCTCTCCATATGCTCTT (58) ACTGAGGAGGCTGGGAGGCTC (59)	194	62	end of S2 to beginning of S3
4	GATGACGCCATTGCTTTCGCATGA (60) GTGGGAAGCCCATGTGGTCCTG (61)	298	65	end of S3 to S4
5	CATCCACTCAACGACTCCCCAG (62) AATCTCACAGAATTGGCCTCCAAG (63)	249	65	S5 to beginning of the pore
6	TCCATGTGGTACTCCATGTCTGAA (64) GCACGTCACATTGGGGATGTCAT (65)	190	58	end of the pore to beginning of S6
7	GGAATGCTGGGACAGTCTAGCTG (66) TACATATGCATGGATCTTAATCCCAT (67)	203	58	end of S6 to start of C-terminal part
8	AAAGTTTCAGGTGGTGCCCACTCA (68) GAGGCCACAGACACGAATACAGAC (69)	230	65	C- terminal
9	TGGGTAAACCCGCCTCCTTCATTG (70) ACTCTATCTTGGGACCAGCATGAC (71)	306	65	C- terminal
10	TAAGAGCCTGCACTGAAGGAGGA (72) GGGGAGGAAGAAGTGGAAGAGAC (73)	302	65	C- terminal
11	CAGGTCTGTGGCCTGCCGTTTCAT (74) CCTTCCTGTGGGAGTTGAGCTGG (75)	233	65	C- terminal
12	GTTTGCTAGCCTTCTGTTATAGCT (76) GGGAGCGCAGTCCCTCCAGAT (77)	239	62	C- terminal
13	CTTATATATTCCAAACCCTTATCTCA (78) GGTGGGGATCGTTGCTATTGGTT (79)	277	62	C- terminal
14	AACCAATAGCAACGATCCCCACC (80) CTGACTTTGTCAATGGTCACCTGG (81)	303	65	C- terminal after last intron
15	CGGAACCACCCTACAGCTTCCA (82) GGGAGTGGCAGCTCACTCGGGA (83)	210	65	C- terminal after last intron
16	AGGCCACGGTCCTGCCTATCT (84) CCATTGGGGCCGAACACATAATC (85)	236	65	C- terminal after last intron
17	CTTCAGCATCTCCAGGACAGAG (86) AAGGAGGGGTCAGCCAGTGACCT (87)	228	65	C- terminal after the STOP codon

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended

5 claims.

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WHAT IS CLAIMED IS:

1. An isolated nucleic acid or its complement comprising nucleic acid encoding a protein selected from SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:89, SEQ ID NO:91, or SEQ ID NO:96.
2. An isolated nucleic acid or its complement according to claim 1 wherein said nucleic acid comprises a nucleic acid with a sequence of nucleotides 128-2743 of SEQ ID NO:1, nucleotides 19-2634 of SEQ ID NO:6, nucleotides 1-2273 of SEQ ID NO:88, nucleotides 202-2812 of SEQ ID NO:90, or nucleotides 128-2917 of SEQ ID NO:95.
3. An isolated nucleic acid or its complement comprising nucleic acid coding for a mutant human KCNQ2 or KCNQ3 polypeptide which causes benign familial neonatal convulsions (BFNC), juvenile myoclonic epilepsy (JME) or rolandic epilepsy.
4. An isolated nucleic acid according to claim 3 wherein said isolated nucleic acid comprises a mutation which causes BFNC, JME, or rolandic epilepsy wherein said mutation is selected from the group consisting of: a G at nucleotide 978 of SEQ ID NO:1, an A at nucleotide 1043 of SEQ ID NO:1, a T at nucleotide 1094 of SEQ ID NO:1, an A at nucleotide 1125 of SEQ ID NO:1, a T at nucleotide 1469 of SEQ ID NO:1, an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides after nucleotide 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts codon 544 of KCNQ2, a mutation causing a stop codon at or before codon 319 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 524 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 323 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 448 of SEQ ID NO:2, a T at nucleotide 947 of SEQ ID NO:6, and the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
5. An isolated nucleic acid according to claim 3 wherein said isolated nucleic acid encodes a cysteine at codon 284 of SEQ ID NO:2, a threonine at codon 306 of SEQ ID NO:2, a glutamine at codon 333 of SEQ ID NO:2 or a valine at codon 310 of SEQ ID NO:7.



6. A nucleic acid probe which hybridizes specifically to the nucleic acid of claim 1 under high stringency.
7. A nucleic acid probe which hybridizes specifically to a nucleic acid of claim 3 under stringent hybridization conditions wherein said stringent hybridization conditions prevent said nucleic acid probe from hybridizing to nucleic acid defined by SEQ ID NO:1 or SEQ ID NO:6.
8. A nucleic acid probe which hybridizes specifically to a nucleic acid of claim 4 under stringent hybridization conditions wherein said stringent hybridization conditions prevent said nucleic acid probe from hybridizing to nucleic acid defined by SEQ ID NO:1 or SEQ ID NO:6.
9. A method for diagnosing a mutation which causes BFNC, JME, or rolandic epilepsy wherein said method comprises hybridizing a probe of claim 7 to a patient's sample of DNA or RNA under stringent conditions which allow hybridization of said probe to nucleic acid comprising said mutation but prevent hybridization of said probe to wild-type human *KCNQ2* or *KCNQ3* wherein the presence of a hybridization signal indicates the presence of said mutation.
10. A method for diagnosing a mutation which causes BFNC, JME, or rolandic epilepsy wherein said method comprises hybridizing a probe of claim 8 to a patient's sample of DNA or RNA under stringent conditions which allow hybridization of said probe to nucleic acid comprising said mutation but prevent hybridization of said probe to wild-type human *KCNQ2* or *KCNQ3* wherein the presence of a hybridization signal indicates the presence of said mutation.
11. A method according to claim 9 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized.
12. A method according to claim 10 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized.

13. A method according to claim 9 wherein hybridization is performed *in situ*.
14. A method according to claim 10 wherein hybridization is performed *in situ*.
15. A method for diagnosing the presence of a mutation in human *KCNQ2* or *KCNQ3* which causes BFNC, JME, or rolandic epilepsy wherein said method is performed by means which identify the presence of said mutation.
16. The method of claim 15 wherein said mutation is the presence of a G at nucleotide number 978 of SEQ ID NO:1, an A at nucleotide number 1043 of SEQ ID NO:1, a T at nucleotide number 1094 of SEQ ID NO:1, an A at nucleotide number 1125 of SEQ ID NO:1, a T at nucleotide 1469 of SEQ ID NO:1, an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides after nucleotide 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts codon 544 of SEQ ID NO:2, a mutation resulting in a stop codon at or before codon 319 of SEQ ID NO:2, a mutation resulting in a stop codon at or before codon 524 of SEQ ID NO:2, a mutation resulting in a stop codon at or before codon 323 of SEQ ID NO:2, a mutation resulting in a stop codon at or before codon 448 of SEQ ID NO:2, a T at nucleotide 947 of SEQ ID NO:6, or the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
17. The method of claim 15 wherein said means comprises using a single-stranded conformation polymorphism technique to assay for said mutation.
18. The method of claim 17 wherein said mutation is the presence of a G at nucleotide number 978 of SEQ ID NO:1, an A at nucleotide number 1043 of SEQ ID NO:1, a T at nucleotide number 1094 of SEQ ID NO:1, an A at nucleotide number 1125 of SEQ ID NO:1, a T at nucleotide number 1469 of SEQ ID NO:1, an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides following nucleotide number 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts

codon 544 of *KCNQ2*, a mutation causing a stop codon at or before codon 319 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 524 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 323 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 448 of SEQ ID NO:2, a T at 947 of SEQ ID NO:6, or the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.

19. The method of claim 18 wherein said mutation is a T at 947 of SEQ ID NO:6 and further wherein said single-stranded conformation polymorphism technique uses amplified nucleic acid wherein said amplified nucleic acid was prepared using primers of SEQ ID NO:18 and SEQ ID NO:19.
20. The method of claim 15 wherein said means comprises sequencing human *KCNQ2* or *KCNQ3*.
21. The method of claim 20 wherein said mutation is the presence of a G at nucleotide number 978 of SEQ ID NO:1, an A at nucleotide number 1043 of SEQ ID NO:1, a T at nucleotide 1094 of SEQ ID NO:1, an A at nucleotide 1125, a T at nucleotide 1469 of SEQ ID NO:1, an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides after nucleotide 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts codon 544 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 319 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 524 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 323 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 448 of SEQ ID NO:2, a T at nucleotide 947 of SEQ ID NO:6, or the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
22. The method of claim 15 wherein said means comprises performing an RNase assay.
23. The method of claim 22 wherein said mutation is the presence of a G at nucleotide number 978 of SEQ ID NO:1, an A at nucleotide number 1043 of SEQ ID NO:1, a T at nucleotide 1094 of SEQ ID NO:1, an A at nucleotide 1125, a T at nucleotide 1469 of SEQ ID NO:1,

an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides after nucleotide 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts codon 544 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 319 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 524 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 323 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 448 of SEQ ID NO:2, a T at nucleotide 947 of SEQ ID NO:6, or the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.

24. An antibody which binds to a polypeptide of SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:89, or SEQ ID NO:91.
25. An antibody which binds to a mutant human KCNQ2 or mutant human KCNQ3 polypeptide but not to wild-type human KCNQ2 or wild-type human KCNQ3 polypeptide, wherein said mutant polypeptide causes BFNC, JME or rolandic epilepsy.
26. The antibody of claim 25 wherein said mutant polypeptide comprises a cysteine at amino acid residue 284 of SEQ ID NO:2, a threonine at amino acid residue 306 of SEQ ID NO:2, a glutamine at amino acid residue 333 of SEQ ID NO:2, a valine at amino acid residue 310 of SEQ ID NO:7, or wherein said mutant polypeptide is SEQ ID NO:96.
27. A method for diagnosing BFNC, JME, or rolandic epilepsy in a human patient, said method comprising an assay for the presence of mutant KCNQ2 or mutant KCNQ3 polypeptide in said patient by reacting a sample comprising protein from said patient with an antibody of claim 25 wherein the presence of a positive reaction is indicative of BFNC, JME, or rolandic epilepsy.
28. The method of claim 27 wherein said mutant KCNQ2 or mutant KCNQ3 is selected from the group consisting of (a) a KCNQ2 comprising a cysteine at amino acid residue 284 of SEQ ID NO:2, (b) a KCNQ2 comprising a threonine at amino acid residue 306 of SEQ ID NO:2, (c) a KCNQ2 comprising a glutamine at amino acid residue 333 of SEQ ID NO:2, (d)

a KCNQ2 encoded by a DNA of mutated SEQ ID NO:1 wherein said mutated SEQ ID NO:1 is SEQ ID NO:1 altered by an insertion of a GT between nucleotides 975 and 976, (e) a KCNQ2 encoded by a DNA of mutated SEQ ID NO:1 wherein said mutated SEQ ID NO:1 is SEQ ID NO:1 altered by an insertion of a GGGCC following nucleotide 2736 of SEQ ID NO:1, (f) a KCNQ2 encoded by a DNA of mutated SEQ ID NO:1 wherein said mutated SEQ ID NO:1 is SEQ ID NO:1 altered by the deletion of 13 nucleotides consisting of nucleotides 1691-1703, (g) a KCNQ2 comprising amino acid residues 1-318 of SEQ ID NO:2, (h) a KCNQ2 comprising amino acid residues 1-523 of SEQ ID NO:2, (i) a KCNQ2 comprising amino acid residues 1-322 of SEQ ID NO:2, (j) a KCNQ2 comprising amino acid residues 1-448 of SEQ ID NO:2, and (k) a KCNQ3 comprising a valine at amino acid residue 310 of SEQ ID NO:7.

29. The method of claim 27 wherein said antibody is a monoclonal antibody..
30. The method of claim 28 wherein said antibody is a monoclonal antibody.
31. An isolated human KCNQ2 or KCNQ3 polypeptide comprising a mutation which causes BFNC, JME, or rolandic epilepsy.
32. The polypeptide of claim 31 wherein said mutation is a cysteine at amino acid residue 284 of SEQ ID NO:2, a threonine at amino acid residue 306 of SEQ ID NO:2, a glutamine at amino acid residue 333 of SEQ ID NO:2, or a valine at amino acid residue 310 of SEQ ID NO:7.
33. An isolated KCNQ2 polypeptide selected from the group consisting of (a) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a GT insertion between bases 975 and 976, (b) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a 13 base deletion consisting of nucleotides 1691-1703, (c) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a GGGCC insertion following nucleotide 2736, (d) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a T at nucleotide 1094, and (e) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a T at nucleotide 1469.

34. A method for diagnosing BFNC, JME, or rolandic epilepsy in a person wherein said method comprises sequencing KCNQ2 or KCNQ3 polypeptide from said person or sequencing KCNQ2 or KCNQ3 polypeptide synthesized from nucleic acid derived from said person wherein the presence of a cysteine at amino acid residue 284 of KCNQ2, a threonine at amino acid residue 306 of KCNQ2, a glutamine at amino acid residue 333 of KCNQ2, or a valine at amino acid residue 310 of KCNQ3 is indicative of BFNC, JME or rolandic epilepsy.
35. A method for diagnosing BFNC, JME, or rolandic epilepsy in a person wherein said method comprises sequencing KCNQ2 polypeptide from said person or sequencing KCNQ2 polypeptide synthesized from nucleic acid derived from said person wherein the presence of (a) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a GT insertion between bases 975 and 976, (b) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a 13 base deletion consisting of nucleotides 1691-1703, (c) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a GGGCC insertion following nucleotide 2736, (d) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a T at nucleotide 1094, and (e) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a T at nucleotide 1469 is indicative of BFNC, JME or rolandic epilepsy.
36. A cell transfected with the DNA of claim 1.
37. A cell transfected with the DNA of claim 2.
- 
38. A cell transfected with the DNA of claim 3.
39. A method to screen for drugs which are useful in treating or preventing BFNC, JME or rolandic epilepsy, said method comprising:
- a) preparing cells with wild-type *KCNQ2* or wild-type *KCNQ3*;
  - b) placing the cells of step (a) into a bathing solution to measure current;
  - c) measuring an induced K<sup>+</sup> current in the cells of step (b);

- d) preparing cells with mutant *KCNQ2* if *KCNQ2* is used in step (a) or preparing cells with mutant *KCNQ3* if *KCNQ3* is used in step (a);
- e) placing the cells of step (d) into a bathing solution to measure current;
- f) measuring an induced  $K^+$  current in the cells of step (e);
- g) adding a drug to the bathing solution of step (e);
- h) measuring an induced  $K^+$  current in the cells of step (g); and
- i) determining whether the drug resulted in an induced  $K^+$  current more similar to or less similar to the induced  $K^+$  current seen in cells with wild-type *KCNQ2* or *KCNQ3* as compared to the current seen in cells with mutant *KCNQ2* or mutant *KCNQ3* in the absence of said drug,

wherein a drug which results in a current more similar to the current seen in cells with wild-type *KCNQ2* or wild-type *KCNQ3* is useful in treating or preventing BFNC, JME or rolandic epilepsy.

- 40. The method of claim 38 wherein said mutant *KCNQ2* comprises a mutation shown in Table 2, said mutant *KCNQ3* comprises a T at nucleotide 947 of SEQ ID NO:6, or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
- 41. The method of claim 39 wherein said cells are mammalian.
- 42. The method of claim 40 wherein said cells are mammalian.
- 43. The method of claim 39 wherein said cells are CHO cells.
- 44. The method of claim 40 wherein said cells are CHO cells.
- 45. The method according to claim 39 wherein human *KCNQ2* RNA or human *KCNQ3* RNA is used in a transfection step.
- 46. A nucleic acid vector comprising wild-type human *KCNQ2* or *KCNQ3*.
- 47. A nucleic acid vector comprising mutant human *KCNQ2* or *KCNQ3*.

48. The nucleic acid vector of claim 47 wherein said mutant human *KCNQ2* comprises a mutation shown in Table 2, wherein said mutant human *KCNQ3* comprises a T at the nucleotide represented by nucleotide 947 of SEQ ID NO:6, or wherein said mutant human *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
49. A nonhuman, transgenic animal wherein said animal comprises wild-type human *KCNQ2* or wild-type human *KCNQ3*.
50. A nonhuman, transgenic animal wherein said animal comprises mutant human *KCNQ2* or mutant human *KCNQ3*.
51. The animal of claim 56 wherein said mutant human *KCNQ2* comprises a mutation shown in Table 2, said mutant *KCNQ3* comprises a T at nucleotide 947 of SEQ ID NO:6, or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
52. A method to screen for drugs which are useful in treating or preventing BFNC, JME or rolandic epilepsy, said method comprising:
- a) preparing a transgenic animal with wild-type human *KCNQ2* or wild-type human *KCNQ3*;
  - b) measuring an induced  $K^+$  current in the transgenic animal of step (a);
  - c) preparing a transgenic animal with mutant human *KCNQ2* if *KCNQ2* is used in step (a) or with mutant human *KCNQ3* if *KCNQ3* is used in step (a);
  - d) measuring an induced  $K^+$  current in the transgenic animal of step (c);
  - e) administering a drug to the transgenic animal of step (c);
  - f) measuring an induced  $K^+$  current in the drug-treated animal of step (e);
  - g) determining whether the drug resulted in an induced  $K^+$  current more similar to or less similar to the induced  $K^+$  current seen in the transgenic animal with wild-type human *KCNQ2* or wild-type human *KCNQ3* as compared to the current seen in a transgenic animal with mutant human *KCNQ2* or mutant human *KCNQ3* in the absence of said drug,



wherein a drug which results in a current more similar to the current seen in transgenic animals with wild-type human *KCNQ2* or wild-type human *KCNQ3* is useful in treating or preventing BFNC, JME or rolandic epilepsy.

53. The method of claim 52 wherein said mutant human *KCNQ2* comprises a mutation shown in Table 2, said mutant *KCNQ3* comprises a T at nucleotide 947 of SEQ ID NO:6, or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
54. A method for diagnosing a mutation which causes BFNC, JME or rolandic epilepsy comprising sequencing *KCNQ2* or *KCNQ3* in a patient's sample of DNA to determine the presence or absence of mutations which cause BFNC, JME or rolandic epilepsy.
55. The method of claim 54 wherein said mutations are selected from the mutations shown in Table 2, from the presence of a T at nucleotide 947 in SEQ ID NO:6 or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
56. The method of claim 54 wherein said patient's sample of DNA has been amplified.
57. The method of claim 55 wherein said patient's sample of DNA has been amplified.
58. A method for diagnosing a mutation which causes BFNC, JME or rolandic epilepsy wherein said method comprises sequencing a *KCNQ2* gene or a *KCNQ3* gene in a patient's sample of RNA to determine the presence or absence of mutations which cause BFNC, JME or rolandic epilepsy.
59. The method of claim 58 wherein said mutations are selected from the mutations shown in Table 2, the presence of a T at nucleotide 947 of SEQ ID NO:6, or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
60. A method for diagnosing a mutation which causes BFNC, JME or rolandic epilepsy wherein said method comprises determining *KCNQ2* or *KCNQ3* sequence in a patient by preparing

cDNA from RNA taken from said patient and sequencing said cDNA to determine the presence or absence of mutations which cause BFNC, JME or rolandic epilepsy.

61. A method of diagnosing the presence of BFNC, JME or rolandic epilepsy by performing in situ hybridization with a probe specific for *KCNQ2* or *KCNQ3* wherein the presence of only a single copy of either *KCNQ2* or *KCNQ3* indicates the presence of BFNC, JME or rolandic epilepsy.
62. A pair of single-stranded DNA primers for determination of a nucleotide sequence of *KCNQ2* by a polymerase chain reaction, the sequence of said primers being derived from human chromosome 20q13, wherein the use of said primers in a polymerase chain reaction results in the synthesis of DNA having all or part of the sequence of *KCNQ2*.
63. A pair of single-stranded DNA primers of claim 62 wherein said pair is selected from:
  - (a) SEQ ID NO:22 and SEQ ID NO:23,
  - (b) SEQ ID NO:24 and SEQ ID NO:25,
  - (c) SEQ ID NO:27 and SEQ ID NO:28,
  - (d) SEQ ID NO:29 and SEQ ID NO:30,
  - (e) SEQ ID NO:31 and SEQ ID NO:32,
  - (f) SEQ ID NO:33 and SEQ ID NO:34,
  - (g) SEQ ID NO:35 and SEQ ID NO:36,
  - (h) SEQ ID NO:37 and SEQ ID NO:38,
  - (i) SEQ ID NO:39 and SEQ ID NO:40,
  - (j) SEQ ID NO:41 and SEQ ID NO:42,
  - (k) SEQ ID NO:43 and SEQ ID NO:44,
  - (l) SEQ ID NO:46 and SEQ ID NO:47,
  - (m) SEQ ID NO:48 and SEQ ID NO:49,
  - (n) SEQ ID NO:50 and SEQ ID NO:51, or
  - (o) SEQ ID NO:52 and SEQ ID NO:53.
64. A pair of single-stranded DNA primers for determination of a nucleotide sequence of *KCNQ3* by a polymerase chain reaction, the sequence of said primers being derived from

human chromosome 8q24, wherein the use of said primers in a polymerase chain reaction results in the synthesis of DNA having all or part of the sequence of *KCNQ3*.

65. A pair of single-stranded DNA primers of claim 64 wherein said pair is selected from:
- (a) SEQ ID NO:54 and SEQ ID NO:55,
  - (b) SEQ ID NO:56 and SEQ ID NO:57,
  - (c) SEQ ID NO:58 and SEQ ID NO:59,
  - (d) SEQ ID NO:60 and SEQ ID NO:61,
  - (e) SEQ ID NO:62 and SEQ ID NO:63,
  - (f) SEQ ID NO:64 and SEQ ID NO:65,
  - (g) SEQ ID NO:66 and SEQ ID NO:67,
  - (h) SEQ ID NO:68 and SEQ ID NO:69,
  - (i) SEQ ID NO:70 and SEQ ID NO:71,
  - (j) SEQ ID NO:72 and SEQ ID NO:73,
  - (k) SEQ ID NO:74 and SEQ ID NO:75,
  - (l) SEQ ID NO:76 and SEQ ID NO:77,
  - (m) SEQ ID NO:78 and SEQ ID NO:79,
  - (n) SEQ ID NO:80 and SEQ ID NO:81,
  - (o) SEQ ID NO:82 and SEQ ID NO:83,
  - (p) SEQ ID NO:84 and SEQ ID NO:85, or
  - (q) SEQ ID NO:86 and SEQ ID NO:87.
66. An isolated DNA comprising DNA having at least 8 consecutive nucleotides of bases 1244-3232 of SEQ ID NO:1 or at least 8 consecutive nucleotides of SEQ ID NO:6.
67. The isolated DNA of claim 66 wherein said DNA comprises at least 15 consecutive nucleotides of bases 1244-3232 of SEQ ID NO:1 or at least 15 consecutive nucleotides of SEQ ID NO:6.
68. An isolated DNA comprising DNA having at least 8 consecutive nucleotides of any one of SEQ ID NOs:100-129.

69. An isolated DNA comprising DNA having at least 15 consecutive nucleotides of any one of SEQ ID NOs:100-129.
70. An isolated nucleic acid comprising a sequence selected from any one of SEQ ID NOs:100-129.

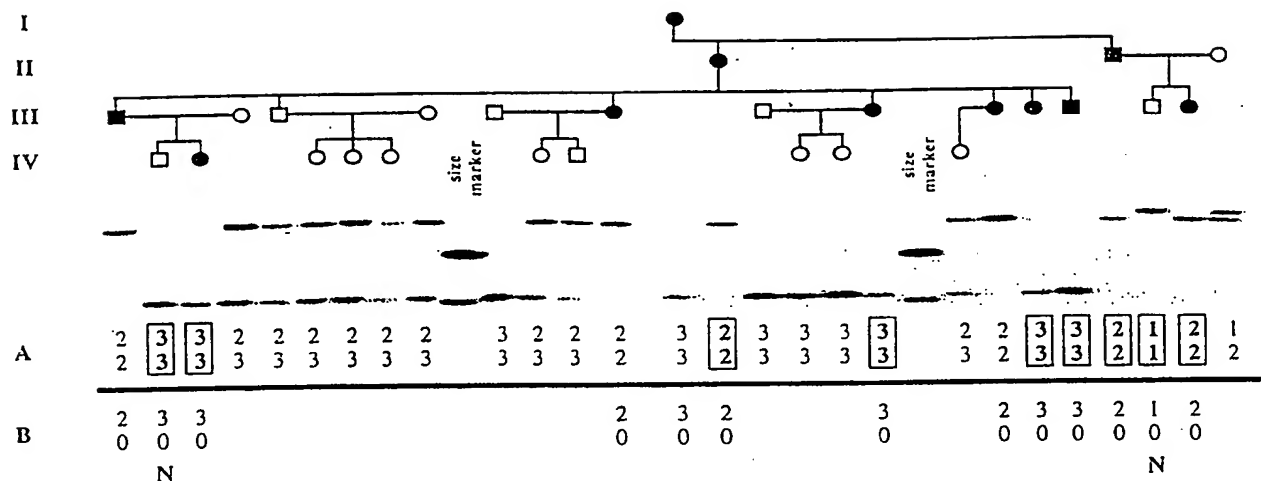
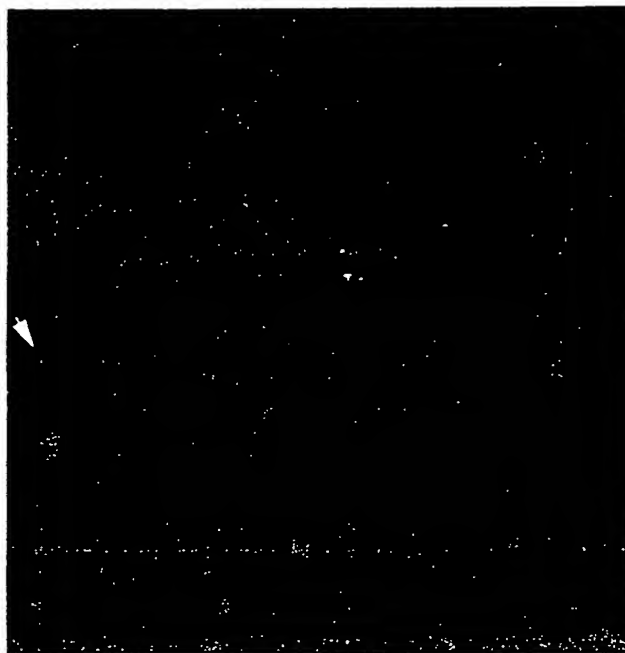
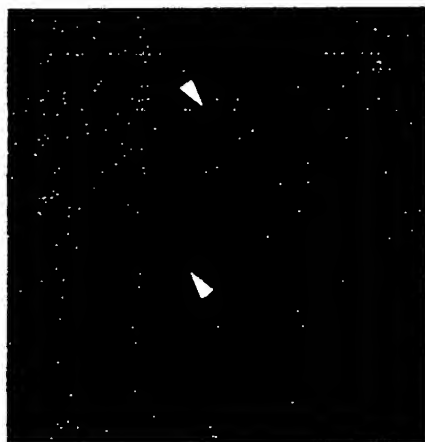


Figure 1



D20S24

Figure 2A



P1-KO9-6b

Figure 2B



P1-KO9-7

Figure 2C

[illegible]

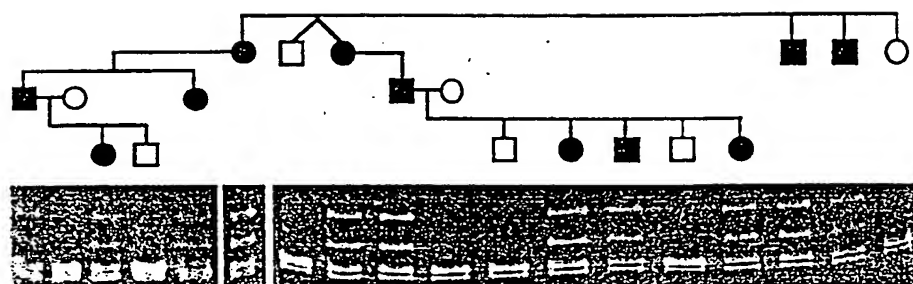


Figure 4



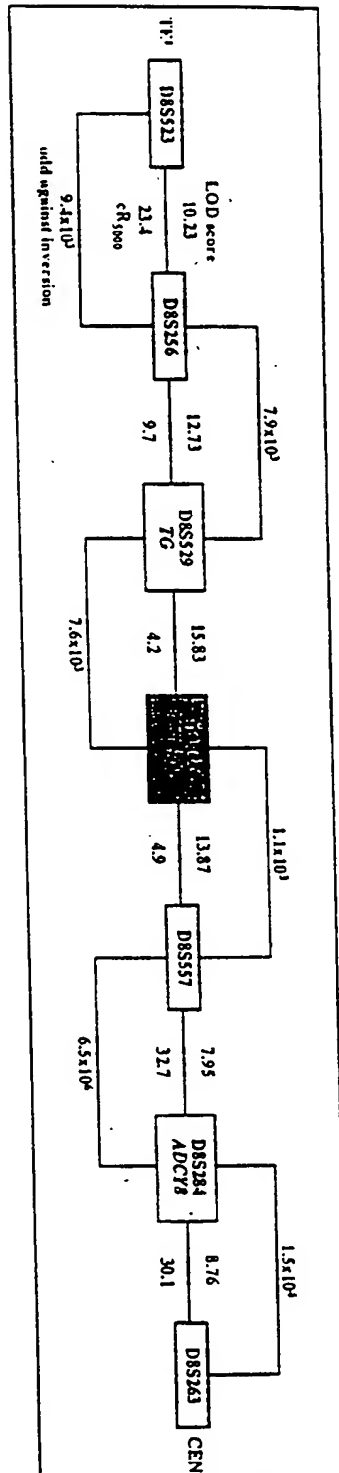


Figure 5

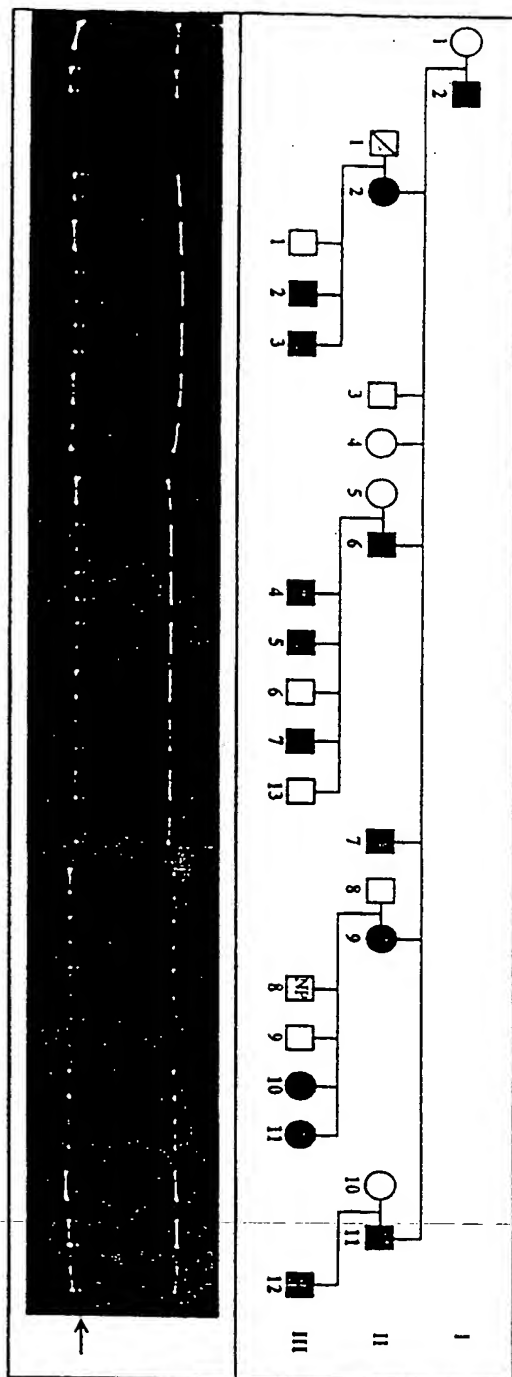


Figure 6

GTTCTCTCTGGTTTTCTCCTGCCTCGTGCTGTCTGTGTTTTCCACCATCAAGGAGTATGAGA  
AGAGCTCGGAGGGGGCCCTCTACATCCTGGTGAGCCCCGAGGGAGGGCGGGGGCTGGAAGTG  
CCCAGGAAGGAGCTGGAGCTGCCTGGGCGTCTGTCTT

Figure 7A

AGGCCTCAAGGTGGCCTCAGCTTTCCTCCCCTGCAGGAAATCGTGACTATCGTGGTGTTTGG  
CGTGAGTACTTTCGTGCGGATCTGGGCCCGCAGGCTGCTGCTGCCGGTACCGTGGCTGGAGGG  
GGCGGCTCAAGTTTGCCCGGAAACCGTTCTGTGTGATTGGTGAGGCCTGGTGGGGGTGGTAT  
TGCTAGAATCAGGGCCAG

Figure 7B

ACATCATGGTGCTCATCGCCTCCATTGCGGTGCTGGCCGCCGGCTCCCAGGGCAACGTCTTT  
GCCACATCTGCGCTCCGGAGCCTGCGCTTCCTGCAGATTCTGCGGATGATCCGCATGGACCG  
GCGGGGAGGCACCTGGAAGCTGCTGGGCTCTGTGGTCTATGCCACA

Figure 7C

TGGTCACTGCCTGGTACATCGGCTTCCTTTGTCTCATCCTGGCCTCGTTCCTGGTGTACTTG  
GCAGAGAAGGGGGAGAACGACCACTTTGACACCTACGCGGATGCACTCTGGTGGGGCCTGGT  
GAGTTGTGGTCATTGTGGTTTTCCCTTTCCCTGCTGATACACCCCTGTCCCTGTGCTGGGAC  
CAGGCTCTCACTGGCTGAGCCTGCTCCAT

Figure 7D

GCAGGCCCTTCGTGTGACTAGAGCCTGCGGTCCCACAGATCACGCTGACCACCATTTGGCTAC  
GGGGACAAGTACCCCCAGACCTGGAACGGCAGGCTCCTTGCGGCAACCTTCACCCCTATCGG  
TGTCTCCTTCTTCGCGCTGCCTGCAGTAAGTCCAGCTGCCCCCTGCCTGCCTTGGAGGGGGAC  
GAGGTCTTGTAGGCTCCCGAGGTGACCACAGGCCCTGGGCACAGTTCCCTAGGT

Figure 7E

ATGGTCTGACCCTGATGAATTGGGGTGTGGGGGGTCCCTGGGGTGTGACCTGACCCTGATGA  
ATTGCAGGGCATCTTGGGGTCTGGGTTTGCCCTGAAGGTTCAAGGAGCAGCACAGGCAGAAGC  
ACTTTGAGAAGAGGCGGAACCCGGCAGCAGGCCTGATCCAGGTGAGTCCAGGTGTCCCCCGG  
GGACCAGCACAGCCCTTGTCTTGGTCCCACCTTGTTGAGGAGTGAGGGCCGC

Figure 7F

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AGCTGTGCAAGCAGAGGGAGGTGTCCCAGGACTCGGGAGGGTGAGACGCTCACTCCCCTCTC  
CTTCTCTTGCCCCAGACTTATCCCCCGCTGAACCAGCTGGAGCTGCTGAGGAACCTCAAGA  
GTAAATCTGGACTCGCTTTCAGGTCAGCTGGGGAGCTCCAGGTGGGGCGGGTGGGCGTCTCA  
GTCTCCTGGGGGCCCCAGCTGCCACAGAAGACACGCCAGGACAG

Figure 7G

CCCAGGACTAACTGTGCTCTCCTCATTTCCAGTAAAGGCAGCCCGTGACAGAGGGCCCCTGTG  
TGGATGCTGCCCCGACGCTCTAGGTACNRCGGAACACRMSSCACGACTGACGGCTGCTGC  
ACGG

Figure 7H

GCAGAGTGACTTCTCTCCCTGTTTTTCTGTCTGTCTGTCTGTCTGTCTGTCGGTTCCCGTGGGAGC  
AGCCAGAAGGTCAGTTTGAAAGATCGTGTCTTCTCCAGCCCCGAGGCGTGGCTGCCAAGGG  
GAAGGGGTCCCCGACGCCCCAGACTGTGAGGCGGTACCCAGCGCCGACCAGAGCCTCGAGG  
ACAGCCCCAGCAAGGTGCCAAGAGCTGGAGCTTCGGGGACCGCAGCCGGGCACGCCAGGCT  
TTCCGCATCAAGGGTGCCGCGTCACGGCAGAACTCAGAAGGGGTGTGGCCGCATCCTCTCCT  
GGTCCATC

Figure 7I

CCCTCACGGCATGTGTCTTCCCCCAGAAGCAAGCCTCCCCGGAGAGGACATTGTGGATGA  
CAAGAGCTGCCCCCTGCGAGTTTGTGACCGAGGACCTGACCCCGGGCCTCAAAGTCAGCATCA  
GAGCCGTGTGGTGAGGCCCTGCCAGCCGGGAGCCTGGGGGAGTGAGGAGGGGCCTCCCCG  
T

Figure 7J

GGTCTCTGGCCCAGGGCTCACAGCCCCACCCACCCCCCTGCAGTGTCATGCGGTTCCCTGGTG  
TCCAAGCGGAAGTTCAAGGAGAGCCTGCGGCCCTACGACGTGATGGACGTCATCGAGCAGTA  
CTCAGCCGGCCACCTGGACATGCTGTCCCGAATTAAGAGCCTGCAGTCCAGGCAAGAGCCCC  
GCCTGCCTGTCCAGCAGGGGACAAG

Figure 7K

CCCAGCCCAGCAGCCCCTTTTGCAGGTCTTGTCCATGGAGAAGAAGCTGGACTTCCTGGTGA  
ATATCTACATGCAGCGGATGGGCATCCCCCGACAGAGACCGAGGCCTACTTTGGGGCCAAA  
GAGCCGGAGCCGGCGCCCGCTACCACAGCCCGGAAGACAGCCGGGAGCATGTGACAGGCA  
CGGCTGCATTGTCAAGATCGTGCGCTCCAGCAGCTCCACGGGCCAGAAGAACTTCTCGGCGC  
CCCCGGCCGCGCCCCCTGTCCAGTGTCCGCCCTCCACCT

Figure 7L

CTCCACGGGCCAGAAGAACTTCTCGGCGCCCCCGGCCGCGCCCCCTGTCCAGTGTCCGCCCT  
CCACCTCCTGGCAGCCACAGAGCCACCCGCGCCAGGGCCACGGCACCTCCCCCGTGGGGGAC  
CACGGCTCCCTGGTGCGCATCCCGCCGCGCCTGCCCACGAGCGGTTCGCTGTCCGCCTACGG  
CGGGGGCAACCGCGCCAGCATGGAGTTCCTGCGGCAGGAGGACACCCCGGGCTGCAGGCCCC  
CCGAGGGGAACCTGCGGGACAGCGACACGTCCATCTCCATC

Figure 7M

TGGAGTTCCTGCGGCAGGAGGACACCCCGAGCTGCAGGCCCCCCGAGGGGACCCTGCGGGAC  
AGCGACACGTCCATCTCCATCCCGTCCGTGGACCACGAGGAGCTGGAGCGTTCCTTCAGCGG  
CTTCAGCATCTCCAGTCCAAGGAGAACCTGGATGCTCTCAACAGCTGCTACGCGGCCGTGG  
CGCCTTGTGCCAAAGTCAGGCCCTACATTGCGGAGGGAGAGTCAGACACC

Figure 7N

GTGGCGCCTTGTGCCAAAGTCAGGCCCTACATTGCGGAGGGAGAGTCAGACACCGACTCCGA  
CCTCTGTACCCCGTGCGGGCCCCCGCCACGCTCGGCCACCGGCGAGGGTCCCTTTGGTGACG  
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CTGAGTGTGGCGGGAAGGGTGGGCCCTGGAGGGGCCCATGTGGGCTGAAGGATGGGGGCTCC  
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GGCTCCAGGAGCAGCGTGCAGGAGCAAGGCTGCCCTGTCCACTCTGCTCAAGGCCGCGGCCG  
ACATCAGCCCGGTGTGAAGAGGGGCGGAGTGATGACGGGTGTTGCAACCTGGCAACAAGCNG  
GGGGTTGNCCAGCCGANCCAAGGGAAGCACANAAGGAAGCTGTNCCCTAAGACCTNCCCNAA  
AGGCGGCCTGTTTGGTAAGACTGCGCCTTGGTCCGGTGGGTTCCGGCAGCAAAAGCGGGTTT  
TGCCGCCCTGTCTGTG

Figure 7O

GGCGACGTGGAGCAAGTCACCTTGGCGCTCGGGGCCGGAGCCGACAAAGACGGGACCCTGCT  
GCTGGAGGGCGGCGGCCGCGACGAGGGGCAGCGGAGGACCCCGCAGGGGCATCGGGCTCCTGG  
CCAAGACCCCGCTGAGCCGCCAGTCAAGAGAAACAACGCCAAGTACCGGCGCATCCAAACT  
TTGATCTACGACGCCCTGGAGAGACCGCGGGGCTGGGCGCTGCTTTACCACGCGTTGGT

Figure 8A

aacttctctcacattgttttatTTaactgggatgattgtttccgcctgccttgcaggtttgt  
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CTGACCACATTCAAGGAGTATGAGACTGTCTCGGGAGACTGGCTTCTGTTACTGgtaagatt  
gcattctggggtaaatgcttctggttgggcttccagagtgatgaaaaggaggttgccttgg  
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Figure 8B

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Figure 8C

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Figure 8D

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Figure 8E

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Figure 8F

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Figure 8G

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Figure 8H

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Figure 8I



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Figure 8J

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Figure 8K

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Figure 8L

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Figure 8M

Figure 8N

Figure 80

## SEQUENCE LISTING

- <110> Leppert, Mark F.  
Singh, Nanda  
Charlier, Carole
- <120> KCNQ2 AND KCNQ3 - POTASSIUM CHANNEL GENES WHICH ARE  
MUTATED IN BENIGN FAMILIAL NEONATAL CONVULSIONS (BFNC)  
AND OTHER EPILEPSIES
- <130> 2323-134
- <140> U.S.  
<141> 1998-10-23
- <150> 60/063,147  
<151> 1997-10-24
- <160> 129
- <170> PatentIn Ver. 2.0
- <210> 1  
<211> 3232  
<212> DNA  
<213> Homo sapiens
- <220>  
<221> CDS  
<222> (128)..(2743)
- <220>  
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<222> (975)..(976)  
<223> There is an insertion of a GT between nucleotides  
975 and 976 in kindred K1504.
- <220>  
<221> mutation  
<222> (978)  
<223> The mutation A to G occurs at this base in kindred  
K3904.
- <220>  
<221> mutation  
<222> (1043)  
<223> The mutation G to A occurs at this base in kindred  
K1705.
- <220>  
<221> mutation  
<222> (1691)..(1703)  
<223> The thirteen nucleotides from 1691-1703 are  
deleted in kindred K3369.

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 <221> allele  
 <222> (1039)  
 <223> This polymorphism of C to T was seen in 7.0% of  
 the control population.

<220>  
 <221> allele  
 <222> (1846)  
 <223> This polymorphism of C to T was seen in 0.57% of  
 the control population.

<220>  
 <221> mutation  
 <222> (1469)  
 <223> The mutation C to T occurs at this base in kindred  
 K1525.

<220>  
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 <222> (1094)  
 <223> The mutation C to T occurs at this base in kindred  
 K4443.

<220>  
 <221> mutation  
 <222> (1125)  
 <223> The mutation G to A occurs at this base in kindred  
 K4516.

<220>  
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 <222> (2736)..(2737)  
 <223> There is an insertion of GGGCC between these two  
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 aggcacc atg gtg cag aag tcg cgc aac ggc ggc gta tac ccc ggc ccg 169  
 Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Pro  
 1 5 10  
 agc ggg gag aag aag ctg aag gtg ggc ttc gtg ggg ctg gac ccc ggc 217  
 Ser Gly Glu Lys Lys Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly  
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 gcg ccc gac tcc acc cgg gac ggg gcg ctg ctg atc gcc ggc tcc gag 265  
 Ala Pro Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu  
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Ala Pro Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala	
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ggc gcc ggg aag ccc ccc aag cgc aac gcc ttc tac cgc aag ctg cag	361
Gly Ala Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln	
65 70 75	
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Asn Phe Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile	
80 85 90	
tac cac gcc tac gtg ttc ctc ctg gtt ttc tcc tgc ctc gtg ctg tct	457
Tyr His Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser	
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Val Phe Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu	
115 120 125	
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Tyr Ile Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe	
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Gly Arg Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met	
160 165 170	
gtg ctc atc gcc tcc att gcg gtg ctg gcc gcc ggc tcc cag ggc aac	697
Val Leu Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn	
175 180 185 190	
gtc ttt gcc aca tct gcg ctc cgg agc ctg cgc ttc ctg cag att ctg	745
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cgg atg atc cgc atg gac cgg cgg gga ggc acc tgg aag ctg ctg ggc	793
Arg Met Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly	
210 215 220	
tct gtg gtc tat gcc cac agc aag gag ctg gtc act gcc tgg tac atc	841
Ser Val Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile	
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Gly Phe Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu	
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Lys Gly Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp	
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Gly Leu Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln	
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Thr Trp Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val	
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Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu	
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aag gtt cag gag cag cac agg cag aag cac ttt gag aag agg cgg aac	1129
Lys Val Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn	
320 325 330	
ccg gca gca ggc ctg atc cag tgc gcc tgg aga ttc tac gcc acc aac	1177
Pro Ala Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn	
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Leu Ser Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr	
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Val Thr Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser	
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Arg Leu Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys	
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Ser Lys Ser Gly Leu Ala Phe Arg Lys Asp Pro Pro Pro Glu Pro Ser	
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cca agt aaa ggc agc ccg tgc aga ggg ccc ctg tgt gga tgc tgc ccc	1417
Pro Ser Lys Gly Ser Pro Cys Arg Gly Pro Leu Cys Gly Cys Cys Pro	
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Gly Arg Ser Ser Gln Lys Val Ser Leu Lys Asp Arg Val Phe Ser Ser	
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ccc cga ggc gtg gct gcc aag ggg aag ggg tcc ccg cag gcc cag act	1513
Pro Arg Gly Val Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr	
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Val Arg Arg Ser Pro Ser Ala Asp Gln Ser Leu Glu Asp Ser Pro Ser	
465 470 475	

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Lys Val Pro Lys Ser Trp Ser Phe Gly Asp Arg Ser Arg Ala Arg Gln	
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Ala Phe Arg Ile Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala	
495 500 505 510	
agc ctc ccc gga gag gac att gtg gat gac aag agc tgc ccc tgc gag	1705
Ser Leu Pro Gly Glu Asp Ile Val Asp Asp Lys Ser Cys Pro Cys Glu	
515 520 525	
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Phe Val Thr Glu Asp Leu Thr Pro Gly Leu Lys Val Ser Ile Arg Ala	
530 535 540	
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His Leu Asp Met Leu Ser Arg Ile Lys Ser Leu Gln Ser Arg Val Asp	
575 580 585 590	
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Gln Ile Val Gly Arg Gly Pro Ala Ile Thr Asp Lys Asp Arg Thr Lys	
595 600 605	
ggc ccg gcc gag gcg gag ctg ccc gag gac ccc agc atg atg gga cgg	1993
Gly Pro Ala Glu Ala Glu Leu Pro Glu Asp Pro Ser Met Met Gly Arg	
610 615 620	
ctc ggg aag gtg gag aag cag gtc ttg tcc atg gag aag aag ctg gac	2041
Leu Gly Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu Asp	
625 630 635	
ttc ctg gtg aat atc tac atg cag cgg atg ggc atc ccc ccg aca gag	2089
Phe Leu Val Asn Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu	
640 645 650	
acc gag gcc tac ttt ggg gcc aaa gag ccg gag ccg gcg ccg ccg tac	2137
Thr Glu Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr	
655 660 665 670	
cac agc ccg gaa gac agc cgg gag cat gtc gac agg cac ggc tgc att	2185
His Ser Pro Glu Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile	
675 680 685	
gtc aag atc gtg cgc tcc agc agc tcc acg ggc cag aag aac ttc tcg	2233
Val Lys Ile Val Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser	
690 695 700	



gcg ccc ccg gcc gcg ccc cct gtc cag tgt ccg ccc tcc acc tcc tgg 2281  
 Ala Pro Pro Ala Ala Pro Pro Val Gln Cys Pro Pro Ser Thr Ser Trp  
 705 710 715

cag cca cag agc cac ccg cgc cag ggc cac ggc acc tcc ccc gtg ggg 2329  
 Gln Pro Gln Ser His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly  
 720 725 730

gac cac ggc tcc ctg gtg cgc atc ccg ccg ccg cct gcc cac gag cgg 2377  
 Asp His Gly Ser Leu Val Arg Ile Pro Pro Pro Pro Ala His Glu Arg  
 735 740 745 750

tcg ctg tcc gcc tac ggc ggg ggc aac cgc gcc agc atg gag ttc ctg 2425  
 Ser Leu Ser Ala Tyr Gly Gly Gly Asn Arg Ala Ser Met Glu Phe Leu  
 755 760 765

cgg cag gag gac acc ccg ggc tgc agg ccc ccc gag ggg aac ctg cgg 2473  
 Arg Gln Glu Asp Thr Pro Gly Cys Arg Pro Pro Glu Gly Asn Leu Arg  
 770 775 780

gac agc gac acg tcc atc tcc atc ccg tcc gtg gac cac gag gag ctg 2521  
 Asp Ser Asp Thr Ser Ile Ser Ile Pro Ser Val Asp His Glu Glu Leu  
 785 790 795

gag cgt tcc ttc agc ggc ttc agc atc tcc cag tcc aag gag aac ctg 2569  
 Glu Arg Ser Phe Ser Gly Phe Ser Ile Ser Gln Ser Lys Glu Asn Leu  
 800 805 810

gat gct ctc aac agc tgc tac gcg gcc gtg gcg cct tgt gcc aaa gtc 2617  
 Asp Ala Leu Asn Ser Cys Tyr Ala Ala Val Ala Pro Cys Ala Lys Val  
 815 820 825 830

agg ccc tac att gcg gag gga gag tca gac acc gac tcc gac ctc tgt 2665  
 Arg Pro Tyr Ile Ala Glu Gly Glu Ser Asp Thr Asp Ser Asp Leu Cys  
 835 840 845

acc ccg tgc ggg ccc ccg cca cgc tcg gcc acc ggc gag ggt ccc ttt 2713  
 Thr Pro Cys Gly Pro Pro Pro Arg Ser Ala Thr Gly Glu Gly Pro Phe  
 850 855 860

ggt gac gtg ggc tgg gcc ggg ccc agg aag tgaggcggcg ctgggccagt 2763  
 Gly Asp Val Gly Trp Ala Gly Pro Arg Lys  
 865 870

ggacccgccc gcggccctcc tcagcacggt gcctccgagg ttttgaggcg ggaaccctct 2823

ggggcccttt tcttacagta actgagtgtg gcgggaaggg tgggccctgg aggggcccat 2883

gtgggctgaa ggatgggggc tcctggcagt gaccttttac aaaagttatt ttccaacagg 2943

ggctggaggg ctgggcaggg cctgtggctc caggagcagc gtgcaggagc aaggctgccc 3003

tgtccactct gctcaaggcc gcggccgaca tcagcccgggt gtgaagaggg gcggagtgat 3063

7

gacgggtgtt gcaacctggc aacaagcngg gggttgncca gccganccaa gggaagcaca 3123  
 naaggaagct gtncctaag acctnccna aaggcggcct gtttggttaag actgcgcctt 3183  
 ggtccggtgg gttccggcag caaaagcggg ttttgccgcc cctgtcgtg 3232

&lt;210&gt; 2

&lt;211&gt; 872

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 2

Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Pro Ser Gly  
 1 5 10 15

Glu Lys Lys Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Ala Pro  
 20 25 30

Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro  
 35 40 45

Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala  
 50 55 60

Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
 65 70 75 80

Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His  
 85 90 95

Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
 100 105 110

Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile  
 115 120 125

Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
 130 135 140

Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
 145 150 155 160

~~Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu~~  
~~165 170 175~~

Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe  
 180 185 190

Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met  
 195 200 205

Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val  
 210 215 220

Val	Tyr	Ala	His	Ser	Lys	Glu	Leu	Val	Thr	Ala	Trp	Tyr	Ile	Gly	Phe	225	230	235	240
Leu	Cys	Leu	Ile	Leu	Ala	Ser	Phe	Leu	Val	Tyr	Leu	Ala	Glu	Lys	Gly	245	250	255	
Glu	Asn	Asp	His	Phe	Asp	Thr	Tyr	Ala	Asp	Ala	Leu	Trp	Trp	Gly	Leu	260	265	270	
Ile	Thr	Leu	Thr	Thr	Ile	Gly	Tyr	Gly	Asp	Lys	Tyr	Pro	Gln	Thr	Trp	275	280	285	
Asn	Gly	Arg	Leu	Leu	Ala	Ala	Thr	Phe	Thr	Leu	Ile	Gly	Val	Ser	Phe	290	295	300	
Phe	Ala	Leu	Pro	Ala	Gly	Ile	Leu	Gly	Ser	Gly	Phe	Ala	Leu	Lys	Val	305	310	315	320
Gln	Glu	Gln	His	Arg	Gln	Lys	His	Phe	Glu	Lys	Arg	Arg	Asn	Pro	Ala	325	330	335	
Ala	Gly	Leu	Ile	Gln	Ser	Ala	Trp	Arg	Phe	Tyr	Ala	Thr	Asn	Leu	Ser	340	345	350	
Arg	Thr	Asp	Leu	His	Ser	Thr	Trp	Gln	Tyr	Tyr	Glu	Arg	Thr	Val	Thr	355	360	365	
Val	Pro	Met	Tyr	Ser	Ser	Gln	Thr	Gln	Thr	Tyr	Gly	Ala	Ser	Arg	Leu	370	375	380	
Ile	Pro	Pro	Leu	Asn	Gln	Leu	Glu	Leu	Leu	Arg	Asn	Leu	Lys	Ser	Lys	385	390	395	400
Ser	Gly	Leu	Ala	Phe	Arg	Lys	Asp	Pro	Pro	Pro	Glu	Pro	Ser	Pro	Ser	405	410	415	
Lys	Gly	Ser	Pro	Cys	Arg	Gly	Pro	Leu	Cys	Gly	Cys	Cys	Pro	Gly	Arg	420	425	430	
Ser	Ser	Gln	Lys	Val	Ser	Leu	Lys	Asp	Arg	Val	Phe	Ser	Ser	Pro	Arg	435	440	445	
Gly	Val	Ala	Ala	Lys	Gly	Lys	Gly	Ser	Pro	Gln	Ala	Gln	Thr	Val	Arg	450	455	460	
Arg	Ser	Pro	Ser	Ala	Asp	Gln	Ser	Leu	Glu	Asp	Ser	Pro	Ser	Lys	Val	465	470	475	480
Pro	Lys	Ser	Trp	Ser	Phe	Gly	Asp	Arg	Ser	Arg	Ala	Arg	Gln	Ala	Phe	485	490	495	
Arg	Ile	Lys	Gly	Ala	Ala	Ser	Arg	Gln	Asn	Ser	Glu	Glu	Ala	Ser	Leu	500	505	510	

Pro Gly Glu Asp Ile Val Asp Asp Lys Ser Cys Pro Cys Glu Phe Val  
 515 520 525

Thr Glu Asp Leu Thr Pro Gly Leu Lys Val Ser Ile Arg Ala Val Cys  
 530 535 540

Val Met Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu Ser Leu Arg  
 545 550 555 560

Pro Tyr Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu  
 565 570 575

Asp Met Leu Ser Arg Ile Lys Ser Leu Gln Ser Arg Val Asp Gln Ile  
 580 585 590

Val Gly Arg Gly Pro Ala Ile Thr Asp Lys Asp Arg Thr Lys Gly Pro  
 595 600 605

Ala Glu Ala Glu Leu Pro Glu Asp Pro Ser Met Met Gly Arg Leu Gly  
 610 615 620

Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu Asp Phe Leu  
 625 630 635 640

Val Asn Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu Thr Glu  
 645 650 655

Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr His Ser  
 660 665 670

Pro Glu Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile Val Lys  
 675 680 685

Ile Val Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser Ala Pro  
 690 695 700

Pro Ala Ala Pro Pro Val Gln Cys Pro Pro Ser Thr Ser Trp Gln Pro  
 705 710 715 720

Gln Ser His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly Asp His  
 725 730 735

Gly Ser Leu Val Arg Ile Pro Pro Pro Pro Ala His Glu Arg Ser Leu  
 740 745 750

Ser Ala Tyr Gly Gly Gly Asn Arg Ala Ser Met Glu Phe Leu Arg Gln  
 755 760 765

Glu Asp Thr Pro Gly Cys Arg Pro Pro Glu Gly Asn Leu Arg Asp Ser  
 770 775 780

Asp Thr Ser Ile Ser Ile Pro Ser Val Asp His Glu Glu Leu Glu Arg  
 785 790 795 800

10

Ser Phe Ser Gly Phe Ser Ile Ser Gln Ser Lys Glu Asn Leu Asp Ala  
                             805                            810                            815

Leu Asn Ser Cys Tyr Ala Ala Val Ala Pro Cys Ala Lys Val Arg Pro  
                             820                            825                            830

Tyr Ile Ala Glu Gly Glu Ser Asp Thr Asp Ser Asp Leu Cys Thr Pro  
                             835                            840                            845

Cys Gly Pro Pro Pro Arg Ser Ala Thr Gly Glu Gly Pro Phe Gly Asp  
                             850                            855                            860

Val Gly Trp Ala Gly Pro Arg Lys  
                             865                            870

&lt;210&gt; 3

&lt;211&gt; 807

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 3

Met Asp Glu Glu Ser Gly Ser Ser Val Ser Met Trp Leu Thr Met Arg  
       1                            5                            10                            15

Lys Leu Ser Pro Val Ala Met Val Ser Arg Ser Gln Lys Lys Thr Thr  
                             20                            25                            30

Asp Gln Ala Ala Pro Ser Asp Glu Gln Gln Glu Ala Gly Ser Ser Ser  
                             35                            40                            45

Ala Ile Gly Gln Glu Ser Arg Lys Thr Val Val Phe Gln Glu Pro Asp  
                             50                            55                            60

Ile Gly Phe Pro Ser Glu His Asp Gln Leu Thr Thr Leu His Asp Ser  
                             65                            70                            75                            80

Glu Glu Gly Asn Arg Lys Met Ser Leu Val Gly Lys Pro Leu Thr Tyr  
                             85                            90                            95

Lys Asn Tyr Arg Thr Asp Gln Arg Phe Arg Arg Met Gln Asn Lys Met  
                             100                            105                            110

His Asn Phe Leu Glu Arg Pro Arg Gly Trp Lys Ala Ala Thr Tyr His  
                             115                            120                            125

Leu Ala Val Leu Phe Met Val Leu Met Cys Leu Ala Leu Ser Val Phe  
                             130                            135                            140

Ser Thr Met Pro Asp Phe Glu Val Asn Ala Thr Ile Val Leu Tyr Tyr  
                             145                            150                            155                            160

Leu Glu Ile Val Phe Val Ile Trp Leu Ala Thr Glu Tyr Ile Cys Arg  
                             165                            170                            175

11

Val Trp Ser Ala Gly Cys Arg Ser Arg Tyr Arg Gly Ile Ser Gly Arg  
 180 185 190  
 Ile Arg Phe Ala Thr Ser Ala Tyr Cys Val Ile Asp Ile Ile Val Ile  
 195 200 205  
 Leu Ala Ser Ile Thr Val Leu Cys Ile Gly Ala Thr Gly Gln Val Phe  
 210 215 220  
 Ala Ala Ser Ala Ile Arg Gly Leu Arg Phe Phe Gln Leu Arg Met Leu  
 225 230 235 240  
 Arg Ile Asp Arg Arg Ala Gly Thr Trp Lys Leu Leu Gly Ser Val Val  
 245 250 255  
 Trp Ala His Arg Gln Glu Leu Leu Thr Thr Val Tyr Ile Gly Phe Leu  
 260 265 270  
 Gly Leu Ile Phe Ser Ser Phe Leu Val Tyr Leu Cys Glu Lys Asn Thr  
 275 280 285  
 Asn Asp Lys Tyr Gln Thr Phe Ala Asp Ala Leu Trp Trp Gly Val Ile  
 290 295 300  
 Thr Leu Ser Thr Val Gly Tyr Gly Asp Lys Thr Pro Glu Thr Trp Pro  
 305 310 315 320  
 Gly Lys Ile Ile Ala Ala Phe Cys Ala Leu Leu Gly Ile Ser Phe Phe  
 325 330 335  
 Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val Gln  
 340 345 350  
 Gln His Gln Arg Gln Lys His Leu Ile Arg Arg Arg Val Pro Ala Ala  
 355 360 365  
 Lys Leu Ile Gln Cys Leu Trp Arg His Tyr Ser Ala Ala Pro Glu Ser  
 370 375 380  
 Thr Ser Leu Ala Thr Trp Lys Ile His Leu Ala Arg Glu Leu Pro Pro  
 385 390 395 400  
 Ile Val Lys Leu Thr Pro Leu Gly Ser Asn Asn Ala Thr Gly Leu Ile  
 405 410 415  
 Asn Arg Leu Arg Gln Ser Thr Lys Arg Thr Pro Asn Leu Asn Asn Gln  
 420 425 430  
 Asn Leu Ala Val Asn Ser Gln Ala Thr Ser Lys Asn Leu Ser Val Pro  
 435 440 445  
 Arg Val Arg Val His Asp Thr Ile Ser Leu Val Ser Thr Ser Asp Ile  
 450 455 460

Ser Glu Ile Glu Gln Leu Gly Ala Leu Gly Phe Ser Leu Gly Trp Lys  
 465 470 475 480  
 Ser Lys Ser Lys Tyr Gly Gly Ser Lys Lys Ala Thr Asp Asp Ser Val  
 485 490 495  
 Leu Gln Ser Arg Met Leu Ala Pro Ser Asn Ala His Leu Asp Asp Met  
 500 505 510  
 Arg Arg Arg Ser Arg Arg Ser Ala Ser Leu Cys Arg Val Val Asn Thr  
 515 520 525  
 Gly Gln His Leu Arg Pro Leu Gln Pro Arg Ser Thr Leu Ser Asp Ser  
 530 535 540  
 Asp Val Ile Gly Asp Tyr Ser Leu Met Met Ala Pro Ile Tyr Gln Trp  
 545 550 555 560  
 Cys Glu Gln Met Val Gln Arg Asn Ser Thr Pro Gly Glu Asp Gly Val  
 565 570 575  
 Trp Ser Gln Leu Ser Gln Leu Ser Gln Leu Thr Thr Cys Ala Thr Arg  
 580 585 590  
 Arg Thr Glu Asp Ile Ser Asp Gly Asp Glu Glu Glu Ala Val Gly Tyr  
 595 600 605  
 Gln Pro Gln Thr Ile Glu Glu Phe Thr Pro Ala Leu Lys Asn Cys Val  
 610 615 620  
 Arg Ala Ile Arg Arg Ile Gln Leu Leu Val Ala Arg Lys Lys Phe Lys  
 625 630 635 640  
 Glu Ala Leu Lys Pro Tyr Asp Val Lys Asp Val Ile Glu Gln Tyr Ser  
 645 650 655  
 Ala Gly His Val Asp Leu Gln Ser Arg Val Lys Thr Val Gln Ala Lys  
 660 665 670  
 Leu Asp Phe Ile Cys Gly Lys Asn Ile Glu Lys Ile Glu Pro Lys Ile  
 675 680 685  
 Ser Met Phe Thr Arg Ile Ala Thr Leu Glu Thr Thr Val Gly Lys Met  
 690 695 700  
 Asp Lys Lys Leu Asp Leu Met Val Glu Met Leu Met Gly Arg Gln Ala  
 705 710 715 720  
 Ser Gln Arg Val Phe Ser Gln Asn Thr Ser Pro Arg Gly Glu Phe Ser  
 725 730 735  
 Glu Pro Thr Ser Ala Arg Gln Asp Leu Thr Arg Ser Arg Arg Ser Met  
 740 745 750

13

Val Ser Thr Asp Met Glu Met Tyr Thr Ala Arg Ser His Ser Pro Gly  
755 760 765

Tyr His Gly Asp Ala Arg Pro Ile Ile Ala Gln Ile Asp Ala Asp Asp  
770 775 780

Asp Asp Glu Asp Glu Asn Val Phe Asp Asp Ser Thr Pro Leu Asn Asn  
785 790 795 800

Gly Pro Gly Thr Ser Ser Cys  
805

<210> 4

<211> 677

<212> PRT

<213> Homo sapiens

<400> 4

Met Ala Ala Ala Ser Ser Pro Pro Arg Ala Glu Arg Lys Arg Trp Gly  
1 5 10 15

Trp Gly Arg Leu Pro Gly Ala Arg Arg Gly Ser Ala Gly Leu Ala Lys  
20 25 30

Lys Cys Pro Phe Ser Leu Glu Leu Ala Glu Gly Gly Pro Ala Gly Gly  
35 40 45

Ala Leu Tyr Ala Pro Ile Ala Pro Gly Ala Pro Gly Pro Ala Pro Pro  
50 55 60

Ala Ser Pro Ala Ala Pro Ala Ala Pro Pro Val Ala Ser Asp Leu Gly  
65 70 75 80

Pro Arg Pro Pro Val Ser Asp Leu Pro Arg Val Ser Ile Tyr Ser Thr  
85 90 95

Arg Arg Pro Val Leu Ala Arg Thr His Val Gln Gly Arg Val Tyr Asn  
100 105 110

Phe Leu Glu Arg Pro Thr Gly Trp Lys Cys Phe Val Tyr His Phe Ala  
115 120 125

Val Phe Leu Ile Val Leu Val Cys Leu Ile Phe Ser Val Leu Ser Thr  
130 135 140

Ile Glu Gln Tyr Ala Ala Leu Ala Thr Gly Thr Leu Phe Trp Met Glu  
145 150 155 160

Ile Val Leu Val Val Phe Phe Gly Thr Glu Tyr Val Val Arg Leu Trp  
165 170 175

Ser Ala Gly Cys Arg Ser Lys Tyr Val Gly Leu Trp Gly Arg Leu Arg  
180 185 190



Phe Phe Ala Arg Lys Pro Ile Ser Ile Ile Asp Leu Ile Val Val Val  
 195 200 205  
 Ala Ser Met Val Val Leu Cys Val Gly Ser Lys Gly Gln Val Phe Ala  
 210 215 220  
 Thr Ser Ala Ile Arg Gly Ile Arg Phe Leu Gln Ile Leu Arg Met Leu  
 225 230 235 240  
 His Val Asp Arg Gln Gly Gly Thr Trp Arg Leu Leu Gly Ser Val Val  
 245 250 255  
 Phe Ile His Arg Gln Glu Leu Ile Thr Thr Leu Tyr Ile Gly Phe Leu  
 260 265 270  
 Gly Leu Ile Phe Ser Ser Tyr Phe Val Tyr Leu Ala Glu Lys Asp Ala  
 275 280 285  
 Val Asn Glu Ser Gly Arg Val Glu Phe Gly Ser Tyr Ala Asp Ala Leu  
 290 295 300  
 Trp Trp Gly Val Val Thr Val Thr Thr Ile Gly Tyr Gly Asp Lys Val  
 305 310 315 320  
 Pro Gln Thr Trp Val Gly Lys Thr Ile Ala Ser Cys Phe Ser Val Phe  
 325 330 335  
 Ala Ile Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe  
 340 345 350  
 Ala Leu Lys Val Gln Gln Lys Gln Arg Gln Lys His Phe Asn Arg Gln  
 355 360 365  
 Ile Pro Ala Ala Ala Ser Leu Ile Gln Thr Ala Trp Arg Cys Tyr Ala  
 370 375 380  
 Ala Glu Asn Pro Asp Ser Ser Thr Trp Lys Ile Tyr Ile Arg Lys Ala  
 385 390 395 400  
 Pro Arg Ser His Thr Leu Leu Ser Pro Ser Pro Lys Pro Lys Lys Ser  
 405 410 415  
 Val Val Val Lys Lys Lys Lys Phe Lys Leu Asp Lys Asp Asn Gly Val  
 420 425 430  
 Thr Pro Gly Glu Lys Met Leu Thr Val Pro His Ile Thr Cys Asp Pro  
 435 440 445  
 Pro Glu Glu Arg Arg Leu Asp His Phe Ser Val Asp Gly Tyr Asp Ser  
 450 455 460  
 Ser Val Arg Lys Ser Pro Thr Leu Leu Glu Val Ser Met Pro His Phe  
 465 470 475 480

15

Met Arg Thr Asn Ser Phe Ala Glu Asp Leu Asp Leu Glu Gly Glu Thr  
485 490 495

Leu Leu Thr Pro Ile Thr His Ile Ser Gln Leu Arg Glu His His Arg  
500 505 510

Ala Thr Ile Lys Val Ile Arg Arg Met Gln Tyr Phe Val Ala Lys Lys  
515 520 525

Lys Phe Gln Gln Ala Arg Lys Pro Tyr Asp Val Arg Asp Val Ile Glu  
530 535 540

Gln Tyr Ser Gln Gly His Leu Asn Leu Met Val Arg Ile Lys Glu Leu  
545 550 555 560

Gln Arg Arg Leu Asp Gln Ser Ile Gly Lys Pro Ser Leu Phe Ile Ser  
565 570 575

Val Ser Glu Lys Ser Lys Asp Arg Gly Ser Asn Thr Ile Gly Ala Arg  
580 585 590

Leu Asn Arg Val Glu Asp Lys Val Thr Gln Leu Asp Gln Arg Leu Ala  
595 600 605

Leu Ile Thr Asp Met Leu His Gln Leu Leu Ser Leu His Gly Gly Ser  
610 615 620

Thr Pro Gly Ser Gly Gly Pro Pro Arg Glu Gly Gly Ala His Ile Thr  
625 630 635 640

Gln Pro Cys Gly Ser Gly Gly Ser Val Asp Pro Glu Leu Phe Leu Pro  
645 650 655

Ser Asn Thr Leu Pro Thr Tyr Glu Gln Leu Thr Val Pro Arg Arg Gly  
660 665 670

Pro Asp Glu Gly Ser  
675

&lt;210&gt; 5

&lt;211&gt; 12

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; mutation

&lt;222&gt; (5)

<223> The mutation from G to A occurs at this site in  
kindred K3933.

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (1) .. (5)

16

<220>  
 <221> exon  
 <222> (6)..(11)

<400> 5  
 tgcag tgt cat g

12

<210> 6  
 <211> 2914  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (19)..(2634)

<220>  
 <221> allele  
 <222> (840)  
 <223> The polymorphism of a T to a C at this position  
 has appeared in one individual.

<220>  
 <221> mutation  
 <222> (947)  
 <223> The missense mutation from a G to a T occurs at  
 this position in a BFNC family.

<220>  
 <221> allele  
 <222> (678)  
 <223> This position is polymorphic for C or T.

<220>  
 <221> allele  
 <222> (750)  
 <223> This position is polymorphic for T or C.

<220>  
 <221> allele  
 <222> (1089)  
 <223> This position is polymorphic for G or C.

<220>  
 <221> allele  
 <222> (2598)  
 <223> This position is polymorphic for T or C.

<400> 6  
 ccggcggggg cgaggcag atg ggg ctc aag gcg cgc agg gcg gcg ggg gcg 51  
 Met Gly Leu Lys Ala Arg Arg Ala Ala Gly Ala  
 1 5 10

17

gct ggc ggc ggc ggc gac ggg ggc ggc gga ggc ggc ggg gcg gct aac	99
Ala Gly Gly Gly Gly Asp Gly Gly Gly Gly Gly Gly Gly Ala Ala Asn	
15 20 25	
cca gcc gga ggg gac gcg gcg gcg gcc ggc gac gag gag cgg aaa gtg	147
Pro Ala Gly Gly Asp Ala Ala Ala Gly Asp Glu Glu Arg Lys Val	
30 35 40	
ggg ctg gcg ccc ggc gac gtg gag caa gtc acc ttg gcg ctc ggg gcc	195
Gly Leu Ala Pro Gly Asp Val Glu Gln Val Thr Leu Ala Leu Gly Ala	
45 50 55	
gga gcc gac aaa gac ggg acc ctg ctg ctg gag ggc ggc ggc cgc gac	243
Gly Ala Asp Lys Asp Gly Thr Leu Leu Leu Glu Gly Gly Gly Arg Asp	
60 65 70 75	
gag ggg cag cgg agg acc ccg cag ggc atc ggg ctc ctg gcc aag acc	291
Glu Gly Gln Arg Arg Thr Pro Gln Gly Ile Gly Leu Leu Ala Lys Thr	
80 85 90	
ccg ctg agc cgc cca gtc aag aga aac aac gcc aag tac cgg cgc atc	339
Pro Leu Ser Arg Pro Val Lys Arg Asn Ala Lys Tyr Arg Arg Ile	
95 100 105	
caa act ttg atc tac gac gcc ctg gag aga ccg cgg ggc tgg gcg ctg	387
Gln Thr Leu Ile Tyr Asp Ala Leu Glu Arg Pro Arg Gly Trp Ala Leu	
110 115 120	
ctt tac cac gcg ttg gtg ttc ctg att gtc ctg ggg tgc ttg att ctg	435
Leu Tyr His Ala Leu Val Phe Leu Ile Val Leu Gly Cys Leu Ile Leu	
125 130 135	
gct gtc ctg acc aca ttc aag gag tat gag act gtc tcg gga gac tgg	483
Ala Val Leu Thr Thr Phe Lys Glu Tyr Glu Thr Val Ser Gly Asp Trp	
140 145 150 155	
ctt ctg tta ctg gag aca ttt gct att ttc atc ttt gga gcc gag ttt	531
Leu Leu Leu Leu Glu Thr Phe Ala Ile Phe Ile Phe Gly Ala Glu Phe	
160 165 170	
gct ttg agg atc tgg gct gct gga tgt tgc tgc cga tac aaa ggc tgg	579
Ala Leu Arg Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Lys Gly Trp	
175 180 185	
cgg ggc cga ctg aag ttt gcc agg aag ccc ctg tgc atg ttg gac atc	627
Arg Gly Arg Leu Lys Phe Ala Arg Lys Pro Leu Cys Met Leu Asp Ile	
190 195 200	
ttt gtg ctg att gcc tct gtg cca gtg gtt gct gtg gga aac caa ggc	675
Phe Val Leu Ile Ala Ser Val Pro Val Val Ala Val Gly Asn Gln Gly	
205 210 215	
aat gtt ctg gcc acc tcc ctg cga agc ctg cgc ttc ctg cag atc ctg	723
Asn Val Leu Ala Thr Ser Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu	
220 225 230 235	

cgc atg ctg cgg atg gac cgg aga ggt ggc acc tgg aag ctt ctg ggc	771
Arg Met Leu Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly	
240 245 250	
tca gcc atc tgt gcc cac agc aaa gaa ctc atc acg gcc tgg tac atc	819
Ser Ala Ile Cys Ala His Ser Lys Glu Leu Ile Thr Ala Trp Tyr Ile	
255 260 265	
ggg ttc ctg aca ctc atc ctt tct tca ttt ctt gtc tac ctg gtt gag	867
Gly Phe Leu Thr Leu Ile Leu Ser Ser Phe Leu Val Tyr Leu Val Glu	
270 275 280	
aaa gac gtc cca gag gtg gat gca caa gga gag gag atg aaa gag gag	915
Lys Asp Val Pro Glu Val Asp Ala Gln Gly Glu Glu Met Lys Glu Glu	
285 290 295	
ttt gag acc tat gca gat gcc ctg tgg tgg ggc ctg atc aca ctg gcc	963
Phe Glu Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu Ile Thr Leu Ala	
300 305 310 315	
acc att ggc tat gga gac aag aca ccc aaa acg tgg gaa ggc cgt ctg	1011
Thr Ile Gly Tyr Gly Asp Lys Thr Pro Lys Thr Trp Glu Gly Arg Leu	
320 325 330	
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Ile Ala Ala Thr Phe Ser Leu Ile Gly Val Ser Phe Phe Ala Leu Pro	
335 340 345	
gcg ggc atc ctg ggg tcc ggg ctg gcc ctc aag gtg cag gag caa cac	1107
Ala Gly Ile Leu Gly Ser Gly Leu Ala Leu Lys Val Gln Glu Gln His	
350 355 360	
cgt cag aag cac ttt gag aaa agg agg aag cca gct gct gag ctc att	1155
Arg Gln Lys His Phe Glu Lys Arg Arg Lys Pro Ala Ala Glu Leu Ile	
365 370 375	
cag gct gcc tgg agg tat tat gct acc aac ccc aac agg att gac ctg	1203
Gln Ala Ala Trp Arg Tyr Tyr Ala Thr Asn Pro Asn Arg Ile Asp Leu	
380 385 390 395	
gtg gcg aca tgg aga ttt tat gaa tca gtc gtc tct ttt cct ttc ttc	1251
Val Ala Thr Trp Arg Phe Tyr Glu Ser Val Val Ser Phe Pro Phe Phe	
400 405 410	
agg aaa gaa cag ctg gag gca gca tcc agc caa aag ctg ggt ctc ttg	1299
Arg Lys Glu Gln Leu Glu Ala Ala Ser Ser Gln Lys Leu Gly Leu Leu	
415 420 425	
gat cgg gtt cgc ctt tct aat cct cgt ggt agc aat act aaa gga aag	1347
Asp Arg Val Arg Leu Ser Asn Pro Arg Gly Ser Asn Thr Lys Gly Lys	
430 435 440	

cta ttt acc cct ctg aat gta gat gcc ata gaa gaa agt cct tct aaa	1395
Leu Phe Thr Pro Leu Asn Val Asp Ala Ile Glu Glu Ser Pro Ser Lys	
445 450 455	
 gaa cca aag cct gtt ggc tta aac aat aaa gag cgt ttc cgc acg gcc	1443
Glu Pro Lys Pro Val Gly Leu Asn Asn Lys Glu Arg Phe Arg Thr Ala	
460 465 470 475	
 ttc cgc atg aaa gcc tac gct ttc tgg cag agt tct gaa gat gcc ggg	1491
Phe Arg Met Lys Ala Tyr Ala Phe Trp Gln Ser Ser Glu Asp Ala Gly	
480 485 490	
 aca ggt gac ccc atg gcg gaa gac agg ggc tat ggg aat gac ttc ccc	1539
Thr Gly Asp Pro Met Ala Glu Asp Arg Gly Tyr Gly Asn Asp Phe Pro	
495 500 505	
 atc gaa gac atg atc ccc acc ctg aag gcc gcc atc cga gcc gtc aga	1587
Ile Glu Asp Met Ile Pro Thr Leu Lys Ala Ala Ile Arg Ala Val Arg	
510 515 520	
 att cta caa ttc cgt ctc tat aaa aaa aaa ttc aag gag act ttg agg	1635
Ile Leu Gln Phe Arg Leu Tyr Lys Lys Lys Phe Lys Glu Thr Leu Arg	
525 530 535	
 cct tac gat gtg aag gat gtg att gag cag tat tct gcc ggg cat ctc	1683
Pro Tyr Asp Val Lys Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu	
540 545 550 555	
 gac atg ctt tcc agg ata aag tac ctt cag acg aga ata gat atg att	1731
Asp Met Leu Ser Arg Ile Lys Tyr Leu Gln Thr Arg Ile Asp Met Ile	
560 565 570	
 ttc acc cct gga cct ccc tcc acg cca aaa cac aag aag tct cag aaa	1779
Phe Thr Pro Gly Pro Pro Ser Thr Pro Lys His Lys Lys Ser Gln Lys	
575 580 585	
 ggg tca gca ttc acc ttc cca tcc cag caa tct ccc agg aat gaa cca	1827
Gly Ser Ala Phe Thr Phe Pro Ser Gln Gln Ser Pro Arg Asn Glu Pro	
590 595 600	
 tat gta gcc aga cca tcc aca tca gaa atc gaa gac caa agc atg atg	1875
Tyr Val Ala Arg Pro Ser Thr Ser Glu Ile Glu Asp Gln Ser Met Met	
605 610 615	
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Gly Lys Phe Val Lys Val Glu Arg Gln Val Gln Asp Met Gly Lys Lys	
620 625 630 635	
 ctg gac ttc ctc gtg gat atg cac atg caa cac atg gaa cgg ttg cag	1971
Leu Asp Phe Leu Val Asp Met His Met Gln His Met Glu Arg Leu Gln	
640 645 650	
 gtg cag gtc acg gag tat tac cca acc aag ggc acc tcc tcg cca gct	2019
Val Gln Val Thr Glu Tyr Tyr Pro Thr Lys Gly Thr Ser Ser Pro Ala	
655 660 665	

gaa gca gag aag aag gag gac aac agg tat tcc gat ttg aaa acc atc 2067  
 Glu Ala Glu Lys Lys Glu Asp Asn Arg Tyr Ser Asp Leu Lys Thr Ile  
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atc tgc aac tat tct gag aca ggc ccc ccg gaa cca ccc tac agc ttc 2115  
 Ile Cys Asn Tyr Ser Glu Thr Gly Pro Pro Glu Pro Pro Tyr Ser Phe  
 685 690 695

cac cag gtg acc att gac aaa gtc agc ccc tat ggg ttt ttt gca cat 2163  
 His Gln Val Thr Ile Asp Lys Val Ser Pro Tyr Gly Phe Phe Ala His  
 700 705 710 715

gac cct gtg aac ctg ccc cga ggg gga ccc agt tct gga aag gtt cag 2211  
 Asp Pro Val Asn Leu Pro Arg Gly Gly Pro Ser Ser Gly Lys Val Gln  
 720 725 730

gca act cct cct tcc tca gca aca acg tat gtg gag agg ccc acg gtc 2259  
 Ala Thr Pro Pro Ser Ser Ala Thr Thr Tyr Val Glu Arg Pro Thr Val  
 735 740 745

ctg cct atc ttg act ctt ctc gac tcc cga gtg agc tgc cac tcc cag 2307  
 Leu Pro Ile Leu Thr Leu Leu Asp Ser Arg Val Ser Cys His Ser Gln  
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gct gac ctg cag ggc ccc tac tgc gac cga atc tcc ccc cgg cag aga 2355  
 Ala Asp Leu Gln Gly Pro Tyr Ser Asp Arg Ile Ser Pro Arg Gln Arg  
 765 770 775

cgt agc atc acg cga gac agt gac aca cct ctg tcc ctg atg tgc gtc 2403  
 Arg Ser Ile Thr Arg Asp Ser Asp Thr Pro Leu Ser Leu Met Ser Val  
 780 785 790 795

aac cac gag gag ctg gag agg tct cca agt ggc ttc agc atc tcc cag 2451  
 Asn His Glu Glu Leu Glu Arg Ser Pro Ser Gly Phe Ser Ile Ser Gln  
 800 805 810

gac aga gat gat tat gtg ttc ggc ccc aat ggg ggg tgc agc tgg atg 2499  
 Asp Arg Asp Asp Tyr Val Phe Gly Pro Asn Gly Gly Ser Ser Trp Met  
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 Ile Ser Asp Ser Val Trp Thr Pro Ser Asn Lys Pro Ile  
 860 865 870

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21

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&lt;211&gt; 872

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 7

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 20 25 30  
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 35 40 45  
 Asp Val Glu Gln Val Thr Leu Ala Leu Gly Ala Gly Ala Asp Lys Asp  
 50 55 60  
 Gly Thr Leu Leu Leu Glu Gly Gly Gly Arg Asp Glu Gly Gln Arg Arg  
 65 70 75 80  
 Thr Pro Gln Gly Ile Gly Leu Leu Ala Lys Thr Pro Leu Ser Arg Pro  
 85 90 95  
 Val Lys Arg Asn Asn Ala Lys Tyr Arg Arg Ile Gln Thr Leu Ile Tyr  
 100 105 110  
 Asp Ala Leu Glu Arg Pro Arg Gly Trp Ala Leu Leu Tyr His Ala Leu  
 115 120 125  
 Val Phe Leu Ile Val Leu Gly Cys Leu Ile Leu Ala Val Leu Thr Thr  
 130 135 140  
 Phe Lys Glu Tyr Glu Thr Val Ser Gly Asp Trp Leu Leu Leu Leu Glu  
 145 150 155 160  
 Thr Phe Ala Ile Phe Ile Phe Gly Ala Glu Phe Ala Leu Arg Ile Trp  
 165 170 175  
 Ala Ala Gly Cys Cys Cys Arg Tyr Lys Gly Trp Arg Gly Arg Leu Lys  
 180 185 190  
 Phe Ala Arg Lys Pro Leu Cys Met Leu Asp Ile Phe Val Leu Ile Ala  
 195 200 205



Ser Val Pro Val Val Ala Val Gly Asn Gln Gly Asn Val Leu Ala Thr  
 210 215 220

Ser Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met Leu Arg Met  
 225 230 235 240

Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Ala Ile Cys Ala  
 245 250 255

His Ser Lys Glu Leu Ile Thr Ala Trp Tyr Ile Gly Phe Leu Thr Leu  
 260 265 270

Ile Leu Ser Ser Phe Leu Val Tyr Leu Val Glu Lys Asp Val Pro Glu  
 275 280 285

Val Asp Ala Gln Gly Glu Glu Met Lys Glu Glu Phe Glu Thr Tyr Ala  
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Asp Ala Leu Trp Trp Gly Leu Ile Thr Leu Ala Thr Ile Gly Tyr Gly  
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Asp Lys Thr Pro Lys Thr Trp Glu Gly Arg Leu Ile Ala Ala Thr Phe  
 325 330 335

Ser Leu Ile Gly Val Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly  
 340 345 350

Ser Gly Leu Ala Leu Lys Val Gln Glu Gln His Arg Gln Lys His Phe  
 355 360 365

Glu Lys Arg Arg Lys Pro Ala Ala Glu Leu Ile Gln Ala Ala Trp Arg  
 370 375 380

Tyr Tyr Ala Thr Asn Pro Asn Arg Ile Asp Leu Val Ala Thr Trp Arg  
 385 390 395 400

Phe Tyr Glu Ser Val Val Ser Phe Pro Phe Phe Arg Lys Glu Gln Leu  
 405 410 415

Glu Ala Ala Ser Ser Gln Lys Leu Gly Leu Leu Asp Arg Val Arg Leu  
 420 425 430

Ser Asn Pro Arg Gly Ser Asn Thr Lys Gly Lys Leu Phe Thr Pro Leu  
 435 440 445

Asn Val Asp Ala Ile Glu Glu Ser Pro Ser Lys Glu Pro Lys Pro Val  
 450 455 460

Gly Leu Asn Asn Lys Glu Arg Phe Arg Thr Ala Phe Arg Met Lys Ala  
 465 470 475 480

Tyr Ala Phe Trp Gln Ser Ser Glu Asp Ala Gly Thr Gly Asp Pro Met  
 485 490 495

Ala Glu Asp Arg Gly Tyr Gly Asn Asp Phe Pro Ile Glu Asp Met Ile  
                   500                                  505                                  510

Pro Thr Leu Lys Ala Ala Ile Arg Ala Val Arg Ile Leu Gln Phe Arg  
                   515                                  520                                  525

Leu Tyr Lys Lys Lys Phe Lys Glu Thr Leu Arg Pro Tyr Asp Val Lys  
                   530                                  535                                  540

Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu Asp Met Leu Ser Arg  
 545                                  550                                  555                                  560

Ile Lys Tyr Leu Gln Thr Arg Ile Asp Met Ile Phe Thr Pro Gly Pro  
                                   565                                  570                                  575

Pro Ser Thr Pro Lys His Lys Lys Ser Gln Lys Gly Ser Ala Phe Thr  
                                   580                                  585                                  590

Phe Pro Ser Gln Gln Ser Pro Arg Asn Glu Pro Tyr Val Ala Arg Pro  
                   595                                  600                                  605

Ser Thr Ser Glu Ile Glu Asp Gln Ser Met Met Gly Lys Phe Val Lys  
                   610                                  615                                  620

Val Glu Arg Gln Val Gln Asp Met Gly Lys Lys Leu Asp Phe Leu Val  
 625                                  630                                  635                                  640

Asp Met His Met Gln His Met Glu Arg Leu Gln Val Gln Val Thr Glu  
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Tyr Tyr Pro Thr Lys Gly Thr Ser Ser Pro Ala Glu Ala Glu Lys Lys  
                                   660                                  665                                  670

Glu Asp Asn Arg Tyr Ser Asp Leu Lys Thr Ile Ile Cys Asn Tyr Ser  
                   675                                  680                                  685

Glu Thr Gly Pro Pro Glu Pro Pro Tyr Ser Phe His Gln Val Thr Ile  
                   690                                  695                                  700

Asp Lys Val Ser Pro Tyr Gly Phe Phe Ala His Asp Pro Val Asn Leu  
 705                                  710                                  715                                  720

Pro Arg Gly Gly Pro Ser Ser Gly Lys Val Gln Ala Thr Pro Pro Ser  
                                   725                                  730                                  735

Ser Ala Thr Thr Tyr Val Glu Arg Pro Thr Val Leu Pro Ile Leu Thr  
                                   740                                  745                                  750

Leu Leu Asp Ser Arg Val Ser Cys His Ser Gln Ala Asp Leu Gln Gly  
                   755                                  760                                  765

Pro Tyr Ser Asp Arg Ile Ser Pro Arg Gln Arg Arg Ser Ile Thr Arg  
                   770                                  775                                  780

24

Asp Ser Asp Thr Pro Leu Ser Leu Met Ser Val Asn His Glu Glu Leu  
 785 790 795 800

Glu Arg Ser Pro Ser Gly Phe Ser Ile Ser Gln Asp Arg Asp Asp Tyr  
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Val Phe Gly Pro Asn Gly Gly Ser Ser Trp Met Arg Glu Lys Arg Tyr  
 820 825 830

Leu Ala Glu Gly Glu Thr Asp Thr Asp Thr Asp Pro Phe Thr Pro Ser  
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Trp Thr Pro Ser Asn Lys Pro Ile  
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sequence to demonstrate calculation of homology or  
identity.

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63

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130

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&lt;213&gt; Homo sapiens

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22

&lt;210&gt; 35

&lt;211&gt; 22

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&lt;213&gt; Homo sapiens

&lt;400&gt; 35

cccaggacta actgtgctct cc

22

&lt;210&gt; 36

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&lt;213&gt; Homo sapiens

&lt;400&gt; 36

ccgtgcagca gccgtcagtc c

21

&lt;210&gt; 37

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&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 37

gcagagtgac ttctctccct gtt

23

&lt;210&gt; 38

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 38

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21

&lt;210&gt; 39

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24

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<400> 77  
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21

<210> 78  
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26

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a

1

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23

<210> 81  
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<212> DNA  
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24

<210> 82  
<211> 22  
<212> DNA  
<213> Homo sapiens

<400> 82  
cggaaccacc ctacagcttc ca

22

<210> 83  
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<212> DNA  
<213> Homo sapiens

<400> 83  
gggagtggca gctcactcgg ga

22

<210> 84  
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<400> 84  
aggccacgg tcctgcctat ct

22

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23

<210> 86  
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<400> 86  
cttcagcatc tcccaggaca gag

23

<210> 87  
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<212> DNA  
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<400> 87  
aaggaggggt cagccagtga cct

23



<210> 88  
 <211> 2273  
 <212> DNA  
 <213> Mus musculus

<220>  
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<400> 88  
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 Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Thr Ser Gly  
 1 5 10 15

gaa aan aan ctc aag gtg ggc ttc gtg ggg ctg gac ccc ggc gng ccc 96  
 Glu Xaa Xaa Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Xaa Pro  
 20 25 30

gan tcc aca cgc gac ggc ncn cta ctc atc gcg ggc tcc gag gcc ccc 144  
 Xaa Ser Thr Arg Asp Gly Xaa Leu Leu Ile Ala Gly Ser Glu Ala Pro  
 35 40 45

aag cgc ggc anc ntn ttg agc aag ccg cgg acg ggc ggc gcg gga ncc 192  
 Lys Arg Gly Xaa Xaa Leu Ser Lys Pro Arg Thr Gly Gly Ala Gly Xaa  
 50 55 60

ggg aag ccc ccn aan cgc aac gcc ttc tac cgc aag ctg cag aat ttc 240  
 Gly Lys Pro Xaa Xaa Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
 65 70 75 80

ctc tac aac gtg cta gag cgg ccc cgc ggc tng gcg ttc atc tac cac 288  
 Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Xaa Ala Phe Ile Tyr His  
 85 90 95

gcc tac gtg ttc ctc ctg gtt ttc tcc tgc ctt gtg ctt tct gtg ttt 336  
 Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
 100 105 110

tcc acc atc aag gag tac gag aag agc tct gag ggg gcc ctc tac atc 384  
 Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile  
 115 120 125

ttg gaa atc gtg act atc gtg gta ttc ggt gtt gag tac ttt gtg agg 432  
 Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
 130 135 140

atc tgg gct gca ggc tgc tgt tgc cgg tat cga ggc tgg agg ggc agg 480  
 Ile Trp Ala Ala Gly Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
 145 150 155 160

ctc aag ttt gcc agg aag ccg ttc tgt gtg att gat atc atg gtg ctg 528  
 Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu  
 165 170 175

att gcc tcc att gct gtg ctg gct gct ggt tcc cag ggc aat gtc ttt	576
Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe	
180 185 190	
gcc aca tct gcg ctt cgg agc ttg cgg ttc ttg caa atc ttg cgg atg	624
Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met	
195 200 205	
atc cgt atg gac cgg agg ggt ggc acc tgg aag ctc ttg gga tcg gta	672
Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val	
210 215 220	
gtc tac gct cac agc aag gag ctg gtg act gcc tgg tac att ggc ttc	720
Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe	
225 230 235 240	
ctc tgc ctc atc ctg gcc tca ttt ctg gtg tac ttg gca gaa aag ggt	768
Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly	
245 250 255	
gag aat gac cac ttt gac acc tac gca gat gca ctc tgg tgg ggt ctg	816
Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu	
260 265 270	
atc acc ctg acg acc att ggc tac ggg gac aag tac cct cag acc tgg	864
Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp	
275 280 285	
aac ggg agg ctg ctg gca gcg acc ttt acc ctc att ggt gtc tcg ttc	912
Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe	
290 295 300	
ttt gct ctt ccg gct ggc att ttg gga tcc ggc ttt gcc ctg aaa gtc	960
Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val	
305 310 315 320	
caa gag cag cat cgg caa aaa cac ttt gag aaa cgg cgg aac cct gcg	1008
Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala	
325 330 335	
gca ggt ctg atc cag tct gcc tgg aga ttc tat gct act aac ctc tca	1056
Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser	
340 345 350	
cgc acc gac ctg cac tcc acg tgg cag tac tac gag cgn aca gtc act	1104
Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Xaa Thr Val Thr	
355 360 365	
gtc ccc atg tac agc tca caa act caa acc tat ggg gcc tcc aga ctc	1152
Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu	
370 375 380	
atc cca cct ctg aac cag ctg gag ctg ctg agg aat ctc aag agc aaa	1200
Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys	
385 390 395 400	

tct gga ctc acc ttc agg aag gag cca cag cca gag cca tca cca agc	1248
Ser Gly Leu Thr Phe Arg Lys Glu Pro Gln Pro Glu Pro Ser Pro Ser	
405 410 415	
ccc cga ggc atg gct gcc aag gga aag ggg tct ccc cag gcc cag acg	1296
Pro Arg Gly Met Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr	
420 425 430	
gtc cgg cgg tcc ccc agt gcg gat cag agt ctt gat gac agc ccg agc	1344
Val Arg Arg Ser Pro Ser Ala Asp Gln Ser Leu Asp Asp Ser Pro Ser	
435 440 445	
aag gtg ccc aag agc tgg agc ttt ggt gac cgc agc cgc aca cgc cag	1392
Lys Val Pro Lys Ser Trp Ser Phe Gly Asp Arg Ser Arg Thr Arg Gln	
450 455 460	
gct ttc cgc atc aag ggt gct gca tcc cgg cag aat tca gaa gaa gca	1440
Ala Phe Arg Ile Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala	
465 470 475 480	
agc ctc cct ggg gag gac atc gta gag gac aac aag agc tgt aac tgc	1488
Ser Leu Pro Gly Glu Asp Ile Val Glu Asp Asn Lys Ser Cys Asn Cys	
485 490 495	
gag ttt gtg act gaa gat ctt acc cct ggc ctc aaa gty agc atc aga	1536
Glu Phe Val Thr Glu Asp Leu Thr Pro Gly Leu Lys Xaa Ser Ile Arg	
500 505 510	
gcc gtg tgt gty atg cgg ttc ttg gta tct aag cga aag ttc aaa gag	1584
Ala Val Cys Xaa Met Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu	
515 520 525	
agt ctg cgc cca tat gat gtg atg gac gtc atc gaa cag tac tcg gct	1632
Ser Leu Arg Pro Tyr Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala	
530 535 540	
gga cac ttg gat atg ttg tcc cgc atc aag agc ctg cag acc aga gtg	1680
Gly His Leu Asp Met Leu Ser Arg Ile Lys Ser Leu Gln Thr Arg Val	
545 550 555 560	
gac cag att gtg ggg cgg ggc cca aca ata acg gat aag gat cgc acc	1728
Asp Gln Ile Val Gly Arg Gly Pro Thr Ile Thr Asp Lys Asp Arg Thr	
565 570 575	
aaa ggc cca gcg gaa acg gag ctg ccc gaa gac ccc agc atg atg gga	1776
Lys Gly Pro Ala Glu Thr Glu Leu Pro Glu Asp Pro Ser Met Met Gly	
580 585 590	
cgg ctt ggg aag gtg gag aaa cag gtc ttg tcc atg gaa aag aag ctc	1824
Arg Leu Gly Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu	
595 600 605	

41

gac ttc ttg gtg agc atc tat aca cag aga atg ggc atc caa cca gca 1872  
 Asp Phe Leu Val Ser Ile Tyr Thr Gln Arg Met Gly Ile Gln Pro Ala  
 610 615 620

gag aca gag gcc tat ttt ggg gcc aag gag cct gag ccg gca cca ccc 1920  
 Glu Thr Glu Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro  
 625 630 635 640

tac cac agc cca gag gac agc cgt gac cat gca gac aag cat ggc tgt 1968  
 Tyr His Ser Pro Glu Asp Ser Arg Asp His Ala Asp Lys His Gly Cys  
 645 650 655

atc att aag atc gtc cgc tcc acc agc tct acg ggc cag agg aac tac 2016  
 Ile Ile Lys Ile Val Arg Ser Thr Ser Ser Thr Gly Gln Arg Asn Tyr  
 660 665 670

gca gca ccc cca gcc atc ccc cct gcc cag tgt cct ccc tcc acc tcg 2064  
 Ala Ala Pro Pro Ala Ile Pro Pro Ala Gln Cys Pro Pro Ser Thr Ser  
 675 680 685

tgg cgg cag agc cac cag cgc cat ggc acc tcc cct gtg gga gac cat 2112  
 Trp Arg Gln Ser His Gln Arg His Gly Thr Ser Pro Val Gly Asp His  
 690 695 700

ggc tca ctg gta cgc atc cca cca ctc cct gca cac gag cgg tcg ctg 2160  
 Gly Ser Leu Val Arg Ile Pro Pro Leu Pro Ala His Glu Arg Ser Leu  
 705 710 715 720

tct gcc tac ggt ggg ggc aac aga gcc agt acc gag ttc ttg agg ctg 2208  
 Ser Ala Tyr Gly Gly Gly Asn Arg Ala Ser Thr Glu Phe Leu Arg Leu  
 725 730 735

gag ggc acc cca gcc tgc agg ccc tct gag gct gct gcg gga cag cga 2256  
 Glu Gly Thr Pro Ala Cys Arg Pro Ser Glu Ala Ala Gly Gln Arg  
 740 745 750

cac gtc cat ctc cat cg 2273  
 His Val His Leu His  
 755

&lt;210&gt; 89

&lt;211&gt; 757

&lt;212&gt; PRT

&lt;213&gt; Mus musculus

&lt;400&gt; 89

Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Thr Ser Gly  
 1 5 10 15

Glu Xaa Xaa Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Xaa Pro  
 20 25 30

Xaa Ser Thr Arg Asp Gly Xaa Leu Leu Ile Ala Gly Ser Glu Ala Pro  
 35 40 45

Lys Arg Gly Xaa Xaa Leu Ser Lys Pro Arg Thr Gly Gly Ala Gly Xaa  
 50 55 60  
 Gly Lys Pro Xaa Xaa Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
 65 70 75 80  
 Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Xaa Ala Phe Ile Tyr His  
 85 90 95  
 Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
 100 105 110  
 Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile  
 115 120 125  
 Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
 130 135 140  
 Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
 145 150 155 160  
 Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu  
 165 170 175  
 Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe  
 180 185 190  
 Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met  
 195 200 205  
 Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val  
 210 215 220  
 Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe  
 225 230 235 240  
 Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly  
 245 250 255  
 Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu  
 260 265 270  
 Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp  
 275 280 285  
 Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe  
 290 295 300  
 Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val  
 305 310 315 320  
 Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala  
 325 330 335

43

Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser  
 340 345 350  
 Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Xaa Thr Val Thr  
 355 360 365  
 Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu  
 370 375 380  
 Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys  
 385 390 395 400  
 Ser Gly Leu Thr Phe Arg Lys Glu Pro Gln Pro Glu Pro Ser Pro Ser  
 405 410 415  
 Pro Arg Gly Met Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr  
 420 425 430  
 Val Arg Arg Ser Pro Ser Ala Asp Gln Ser Leu Asp Asp Ser Pro Ser  
 435 440 445  
 Lys Val Pro Lys Ser Trp Ser Phe Gly Asp Arg Ser Arg Thr Arg Gln  
 450 455 460  
 Ala Phe Arg Ile Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala  
 465 470 475 480  
 Ser Leu Pro Gly Glu Asp Ile Val Glu Asp Asn Lys Ser Cys Asn Cys  
 485 490 495  
 Glu Phe Val Thr Glu Asp Leu Thr Pro Gly Leu Lys Xaa Ser Ile Arg  
 500 505 510  
 Ala Val Cys Xaa Met Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu  
 515 520 525  
 Ser Leu Arg Pro Tyr Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala  
 530 535 540  
 Gly His Leu Asp Met Leu Ser Arg Ile Lys Ser Leu Gln Thr Arg Val  
 545 550 555 560  
 Asp Gln Ile Val Gly Arg Gly Pro Thr Ile Thr Asp Lys Asp Arg Thr  
 565 570 575  
 Lys Gly Pro Ala Glu Thr Glu Leu Pro Glu Asp Pro Ser Met Met Gly  
 580 585 590  
 Arg Leu Gly Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu  
 595 600 605  
 Asp Phe Leu Val Ser Ile Tyr Thr Gln Arg Met Gly Ile Gln Pro Ala  
 610 615 620

44

Glu Thr Glu Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro  
 625                                  630                                  635                                  640  
 Tyr His Ser Pro Glu Asp Ser Arg Asp His Ala Asp Lys His Gly Cys  
    645                                  650                                  655  
 Ile Ile Lys Ile Val Arg Ser Thr Ser Ser Thr Gly Gln Arg Asn Tyr  
    660                                  665                                  670  
 Ala Ala Pro Pro Ala Ile Pro Pro Ala Gln Cys Pro Pro Ser Thr Ser  
    675                                  680                                  685  
 Trp Arg Gln Ser His Gln Arg His Gly Thr Ser Pro Val Gly Asp His  
    690                                  695                                  700  
 Gly Ser Leu Val Arg Ile Pro Pro Leu Pro Ala His Glu Arg Ser Leu  
 705                                  710                                  715                                  720  
 Ser Ala Tyr Gly Gly Gly Asn Arg Ala Ser Thr Glu Phe Leu Arg Leu  
    725                                  730                                  735  
 Glu Gly Thr Pro Ala Cys Arg Pro Ser Glu Ala Ala Ala Gly Gln Arg  
    740                                  745                                  750  
 His Val His Leu His  
    755

&lt;210&gt; 90

&lt;211&gt; 2814

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (202) .. (2811)

&lt;400&gt; 90

ctccccaccc gacctcctct ggcccccgagg aggccgcct ttgcctgctt ttggggggggt 60  
 gggcggggag gggcgcgcg atcatggcat tggagttccc gggcttgacg ccgccgccgc 120  
 cgctcgtcc acgtacccca agtgctccta cttcccgagg cagcagcgga gaaggcgaag 180  
 cgcccggtgg gggcgaggca g atg ggt ctc aag gct cgc agg gcg gct ggc 231  
    Met Gly Leu Lys Ala Arg Arg Ala Ala Gly  
    1                                  5                                  10  
 ggg ggc ggc ggc gaa ggg ggc ggc gga ggc ggc ggg gcc gct aac cct 279  
 Gly Gly Gly Gly Glu Gly Gly Gly Gly Gly Gly Gly Ala Ala Asn Pro  
    15                                  20                                  25  
 gct gga ggg gac tcg gcg gtg gcc ggc gac gag gag cgg aaa gtg ggg 327  
 Ala Gly Gly Asp Ser Ala Val Ala Gly Asp Glu Glu Arg Lys Val Gly  
    30                                  35                                  40

45

ctg gcg cca gga gac gtg gag caa gtc acc ttg gcg cta ggg gcc gga	375
Leu Ala Pro Gly Asp Val Glu Gln Val Thr Leu Ala Leu Gly Ala Gly	
45 50 55	
gcc gac aaa gac ggg acc ctg ctg ctg gag ggc ggt ggc cgc gaa gag	423
Ala Asp Lys Asp Gly Thr Leu Leu Leu Glu Gly Gly Gly Arg Glu Glu	
60 65 70	
ggg cag agg agg acc ccg cag ggc atc ggg ctc ctg gca aag acc ccc	471
Gly Gln Arg Arg Thr Pro Gln Gly Ile Gly Leu Leu Ala Lys Thr Pro	
75 80 85 90	
ctg agc cgc cca gtc aag agg aac aac gcc aag tac agg cgc atc caa	519
Leu Ser Arg Pro Val Lys Arg Asn Asn Ala Lys Tyr Arg Arg Ile Gln	
95 100 105	
act ttg atc tat gac gcc ctg gag aga ccg cgg ggc tgg gcg ctg ctc	567
Thr Leu Ile Tyr Asp Ala Leu Glu Arg Pro Arg Gly Trp Ala Leu Leu	
110 115 120	
tac cac gcg ctt gtg ttc ctg att gtc ctg gga tgc ttg att ctg gcc	615
Tyr His Ala Leu Val Phe Leu Ile Val Leu Gly Cys Leu Ile Leu Ala	
125 130 135	
gtg ctc acc acc ttc aag gaa tat gag act gtg tct gga gac tgg ctt	663
Val Leu Thr Thr Phe Lys Glu Tyr Glu Thr Val Ser Gly Asp Trp Leu	
140 145 150	
ttg ctg ctg gaa aca ttt gct att ttc atc ttt gga gct gag ttt gct	711
Leu Leu Leu Glu Thr Phe Ala Ile Phe Ile Phe Gly Ala Glu Phe Ala	
155 160 165 170	
ttg agg atc tgg gct gca gga tgt tgc tgt cga tac aaa ggc tgg cgt	759
Leu Arg Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Lys Gly Trp Arg	
175 180 185	
gga cgg cta aag ttt gcc agg aag ccc ctg tgc atg ttg gac atc ttc	807
Gly Arg Leu Lys Phe Ala Arg Lys Pro Leu Cys Met Leu Asp Ile Phe	
190 195 200	
gta ctg att gcc tct gtg cca gtg gtt gcc gtg gga aac cag ggc aat	855
Val Leu Ile Ala Ser Val Pro Val Val Ala Val Gly Asn Gln Gly Asn	
205 210 215	
gtc ttg gcc acc tcc ctg cga agc ctt cgc ttc ctg cag atc ctg cgc	903
Val Leu Ala Thr Ser Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg	
220 225 230	
atg ctt cga atg gat agg agg ggt ggc acc tgg aag ctc ctg ggc tcg	951
Met Leu Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser	
235 240 245 250	



46

gct atc tgt gcc cac agc aaa gaa ctc atc act gcc tgg tac ata ggc	999
Ala Ile Cys Ala His Ser Lys Glu Leu Ile Thr Ala Trp Tyr Ile Gly	
255 260 265	
ttc ctg aca ctc atc ctt tct tca ttt ctt gtc tac ctg gtg gag aag	1047
Phe Leu Thr Leu Ile Leu Ser Ser Phe Leu Val Tyr Leu Val Glu Lys	
270 275 280	
gat gtg cca gaa atg gat gcc caa gga gag gag atg aag gag gag ttt	1095
Asp Val Pro Glu Met Asp Ala Gln Gly Glu Glu Met Lys Glu Glu Phe	
285 290 295	
gag acc tat gca gat gct ctg tgg tgg ggc ctg atc aca ctg gcc acc	1143
Glu Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu Ile Thr Leu Ala Thr	
300 305 310	
att ggt tat gga gac rag aca cct aaa acc tgg gaa gga cgt ctg att	1191
Ile Gly Tyr Gly Asp Xaa Thr Pro Lys Thr Trp Glu Gly Arg Leu Ile	
315 320 325 330	
gct gcc acc ttt tct tta atc ggc gtc tcc ttt ttt gcc ctt ccg gca	1239
Ala Ala Thr Phe Ser Leu Ile Gly Val Ser Phe Phe Ala Leu Pro Ala	
335 340 345	
ggc atc ctt ggc tca gga ctg gca ctg aag gtt cag gag cag cac cgt	1287
Gly Ile Leu Gly Ser Gly Leu Ala Leu Lys Val Gln Glu Gln His Arg	
350 355 360	
cag aag cac ttt gag aag aga agg aag cca gct gcg gaa ctc atc cag	1335
Gln Lys His Phe Glu Lys Arg Arg Lys Pro Ala Ala Glu Leu Ile Gln	
365 370 375	
gct gcc tgg aga tat tat gct acc aac ccc aac agg ttg gat ctg gtg	1383
Ala Ala Trp Arg Tyr Tyr Ala Thr Asn Pro Asn Arg Leu Asp Leu Val	
380 385 390	
gca acc tgg aga tct tat gaa tca gtt gtc tct ttc cca ttc ttc agg	1431
Ala Thr Trp Arg Ser Tyr Glu Ser Val Val Ser Phe Pro Phe Phe Arg	
395 400 405 410	
aaa gaa caa ctg gaa gca gca gcc agc caa aag ctg ggt ctc ttg gat	1479
Lys Glu Gln Leu Glu Ala Ala Ala Ser Gln Lys Leu Gly Leu Leu Asp	
415 420 425	
cgg gtt cgc ctt tct aat cct cgt ggt agc aat act aaa gga aag cta	1527
Arg Val Arg Leu Ser Asn Pro Arg Gly Ser Asn Thr Lys Gly Lys Leu	
430 435 440	
ttt acc cct ctg aat gta gat gcc ata gaa gaa agc cct tcc aaa gag	1575
Phe Thr Pro Leu Asn Val Asp Ala Ile Glu Glu Ser Pro Ser Lys Glu	
445 450 455	
cca aag cct gtt ggc tta aac aat aaa gag cgt ttc cgc acc gcc ttc	1623
Pro Lys Pro Val Gly Leu Asn Asn Lys Glu Arg Phe Arg Thr Ala Phe	
460 465 470	

cgc atg aaa gcc tac gct ttc tgg cag agw tct gaa gat gct ggg aca	1671
Arg Met Lys Ala Tyr Ala Phe Trp Gln Xaa Ser Glu Asp Ala Gly Thr	
475 480 485 490	
ggc gac ccc atg gca gaa gac agg ggc tat ggg aat gac wtc ctc att	1719
Gly Asp Pro Met Ala Glu Asp Arg Gly Tyr Gly Asn Asp Xaa Leu Ile	
495 500 505	
gma gac atg atc cct rcc ctw aag gct gcc atc cga gct gtc aga att	1767
Xaa Asp Met Ile Pro Xaa Xaa Lys Ala Ala Ile Arg Ala Val Arg Ile	
510 515 520	
cta cag ttc cgt cta tat aaa aaa aag ttc aag gag acg ttg agg cct	1815
Leu Gln Phe Arg Leu Tyr Lys Lys Lys Phe Lys Glu Thr Leu Arg Pro	
525 530 535	
tat gat gtg aaa gat gtg att gag cag tat tcg gcc gga cat ctt gac	1863
Tyr Asp Val Lys Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu Asp	
540 545 550	
atg ctt tcc agg ata aag tac cta cag aca aga ata gat atg att ttc	1911
Met Leu Ser Arg Ile Lys Tyr Leu Gln Thr Arg Ile Asp Met Ile Phe	
555 560 565 570	
acc cct gga cct cca tcc act cca aaa cat aag aag tct cag aaa ggg	1959
Thr Pro Gly Pro Pro Ser Thr Pro Lys His Lys Lys Ser Gln Lys Gly	
575 580 585	
aca gca ttt acc tac cca tcc cag cag tct cca agg aat gaa cca tat	2007
Thr Ala Phe Thr Tyr Pro Ser Gln Gln Ser Pro Arg Asn Glu Pro Tyr	
590 595 600	
gta gcc agg gca gcc aca tca gaa act gaa gac caa agc atg atg ggg	2055
Val Ala Arg Ala Ala Thr Ser Glu Thr Glu Asp Gln Ser Met Met Gly	
605 610 615	
aag ttt gta aaa gtt gaa aga cag gtt cat gac atg ggg aag aaa ctg	2103
Lys Phe Val Lys Val Glu Arg Gln Val His Asp Met Gly Lys Lys Leu	
620 625 630	
gac tyc ctc gtg gac atg cat atg cag cat atg gaa cgc tta cag gta	2151
Asp Xaa Leu Val Asp Met His Met Gln His Met Glu Arg Leu Gln Val	
635 640 645 650	
cat gtc act gag tac tac cca act aar ggg gcc tcc tcc cca gcc gaa	2199
His Val Thr Glu Tyr Tyr Pro Thr Xaa Gly Ala Ser Ser Pro Ala Glu	
655 660 665	
ggg gag aag aaa gaa gac aac agg tac tct gat ttg aaa acc atc atc	2247
Gly Glu Lys Lys Glu Asp Asn Arg Tyr Ser Asp Leu Lys Thr Ile Ile	
670 675 680	

48

tgc aac tac tca gag aca ggg ccc cct gac cct cct tac agc ttc cac	2295
Cys Asn Tyr Ser Glu Thr Gly Pro Pro Asp Pro Pro Tyr Ser Phe His	
685 690 695	
cag gtg ccc atc gac aga gtt ggc cct tac ggg ttt ttt gca cat gat	2343
Gln Val Pro Ile Asp Arg Val Gly Pro Tyr Gly Phe Phe Ala His Asp	
700 705 710	
cct gtg aaa ctg acc cga ggg gga ccc agt tct aca aag gct caa gct	2391
Pro Val Lys Leu Thr Arg Gly Gly Pro Ser Ser Thr Lys Ala Gln Ala	
715 720 725 730	
aac ctt ccc tcc tcg gga agt aca tat gca gag agg ccc aca gtc ctg	2439
Asn Leu Pro Ser Ser Gly Ser Thr Tyr Ala Glu Arg Pro Thr Val Leu	
735 740 745	
ccc atc ttg act ctt ctg gac tca tgt gtg agc tac cac tcc cag aca	2487
Pro Ile Leu Thr Leu Leu Asp Ser Cys Val Ser Tyr His Ser Gln Thr	
750 755 760	
gaa ctg caa ggt ccc tat tcg gac cac atc tcg ccc cgc cag aga cgc	2535
Glu Leu Gln Gly Pro Tyr Ser Asp His Ile Ser Pro Arg Gln Arg Arg	
765 770 775	
agc atc act agg gac agt gat aca cct ctg tcc ctc atg tcc gtc aat	2583
Ser Ile Thr Arg Asp Ser Asp Thr Pro Leu Ser Leu Met Ser Val Asn	
780 785 790	
cac gag gaa ctg gag cgg tct cca agt ggc ttc agc atc tca caa gac	2631
His Glu Glu Leu Glu Arg Ser Pro Ser Gly Phe Ser Ile Ser Gln Asp	
795 800 805 810	
aga gat gat tat gta ttt ggc ccc agt ggg gga tcg agc tgg atg ggg	2679
Arg Asp Asp Tyr Val Phe Gly Pro Ser Gly Gly Ser Ser Trp Met Gly	
815 820 825	
gaa aag cgg tac ctg gct gaa gga gaa acg gac acg gac aca gac ccc	2727
Glu Lys Arg Tyr Leu Ala Glu Gly Glu Thr Asp Thr Asp Thr Asp Pro	
830 835 840	
ttc aca ccc agt ggt tcc atg cct atg tca tcc act ggg gat ggt att	2775
Phe Thr Pro Ser Gly Ser Met Pro Met Ser Ser Thr Gly Asp Gly Ile	
845 850 855	
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 <212> PRT  
 <213> Mus musculus

<400> 91

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 35 40 45

Glu Gln Val Thr Leu Ala Leu Gly Ala Gly Ala Asp Lys Asp Gly Thr  
 50 55 60

Leu Leu Leu Glu Gly Gly Gly Arg Glu Glu Gly Gln Arg Arg Thr Pro  
 65 70 75 80

Gln Gly Ile Gly Leu Leu Ala Lys Thr Pro Leu Ser Arg Pro Val Lys  
 85 90 95

Arg Asn Asn Ala Lys Tyr Arg Arg Ile Gln Thr Leu Ile Tyr Asp Ala  
 100 105 110

Leu Glu Arg Pro Arg Gly Trp Ala Leu Leu Tyr His Ala Leu Val Phe  
 115 120 125

Leu Ile Val Leu Gly Cys Leu Ile Leu Ala Val Leu Thr Thr Phe Lys  
 130 135 140

Glu Tyr Glu Thr Val Ser Gly Asp Trp Leu Leu Leu Leu Glu Thr Phe  
 145 150 155 160

Ala Ile Phe Ile Phe Gly Ala Glu Phe Ala Leu Arg Ile Trp Ala Ala  
 165 170 175

Gly Cys Cys Cys Arg Tyr Lys Gly Trp Arg Gly Arg Leu Lys Phe Ala  
 180 185 190

Arg Lys Pro Leu Cys Met Leu Asp Ile Phe Val Leu Ile Ala Ser Val  
 195 200 205

Pro Val Val Ala Val Gly Asn Gln Gly Asn Val Leu Ala Thr Ser Leu  
 210 215 220

Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met Leu Arg Met Asp Arg  
 225 230 235 240

Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Ala Ile Cys Ala His Ser  
 245 250 255

50

Lys Glu Leu Ile Thr Ala Trp Tyr Ile Gly Phe Leu Thr Leu Ile Leu  
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 Ser Ser Phe Leu Val Tyr Leu Val Glu Lys Asp Val Pro Glu Met Asp  
 275 280 285  
 Ala Gln Gly Glu Glu Met Lys Glu Glu Phe Glu Thr Tyr Ala Asp Ala  
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 Leu Trp Trp Gly Leu Ile Thr Leu Ala Thr Ile Gly Tyr Gly Asp Xaa  
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 Thr Pro Lys Thr Trp Glu Gly Arg Leu Ile Ala Ala Thr Phe Ser Leu  
 325 330 335  
 Ile Gly Val Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly  
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 Leu Ala Leu Lys Val Gln Glu Gln His Arg Gln Lys His Phe Glu Lys  
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 Arg Arg Lys Pro Ala Ala Glu Leu Ile Gln Ala Ala Trp Arg Tyr Tyr  
 370 375 380  
 Ala Thr Asn Pro Asn Arg Leu Asp Leu Val Ala Thr Trp Arg Ser Tyr  
 385 390 395 400  
 Glu Ser Val Val Ser Phe Pro Phe Phe Arg Lys Glu Gln Leu Glu Ala  
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 Ala Ala Ser Gln Lys Leu Gly Leu Leu Asp Arg Val Arg Leu Ser Asn  
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 Pro Arg Gly Ser Asn Thr Lys Gly Lys Leu Phe Thr Pro Leu Asn Val  
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 Asp Ala Ile Glu Glu Ser Pro Ser Lys Glu Pro Lys Pro Val Gly Leu  
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 Asn Asn Lys Glu Arg Phe Arg Thr Ala Phe Arg Met Lys Ala Tyr Ala  
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 Phe Trp Gln Xaa Ser Glu Asp Ala Gly Thr Gly Asp Pro Met Ala Glu  
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 Asp Arg Gly Tyr Gly Asn Asp Xaa Leu Ile Xaa Asp Met Ile Pro Xaa  
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 Lys Lys Lys Phe Lys Glu Thr Leu Arg Pro Tyr Asp Val Lys Asp Val  
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Ile Glu Gln Tyr Ser Ala Gly His Leu Asp Met Leu Ser Arg Ile Lys  
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 Thr Pro Lys His Lys Lys Ser Gln Lys Gly Thr Ala Phe Thr Tyr Pro  
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 Ser Glu Thr Glu Asp Gln Ser Met Met Gly Lys Phe Val Lys Val Glu  
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 Arg Gln Val His Asp Met Gly Lys Lys Leu Asp Xaa Leu Val Asp Met  
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 His Met Gln His Met Glu Arg Leu Gln Val His Val Thr Glu Tyr Tyr  
 645 650 655  
 Pro Thr Xaa Gly Ala Ser Ser Pro Ala Glu Gly Glu Lys Lys Glu Asp  
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 Asn Arg Tyr Ser Asp Leu Lys Thr Ile Ile Cys Asn Tyr Ser Glu Thr  
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 Gly Pro Pro Asp Pro Pro Tyr Ser Phe His Gln Val Pro Ile Asp Arg  
 690 695 700  
 Val Gly Pro Tyr Gly Phe Phe Ala His Asp Pro Val Lys Leu Thr Arg  
 705 710 715 720  
 Gly Gly Pro Ser Ser Thr Lys Ala Gln Ala Asn Leu Pro Ser Ser Gly  
 725 730 735  
 Ser Thr Tyr Ala Glu Arg Pro Thr Val Leu Pro Ile Leu Thr Leu Leu  
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 Asp Ser Cys Val Ser Tyr His Ser Gln Thr Glu Leu Gln Gly Pro Tyr  
 755 760 765  
 Ser Asp His Ile Ser Pro Arg Gln Arg Arg Ser Ile Thr Arg Asp Ser  
 770 775 780  
 Asp Thr Pro Leu Ser Leu Met Ser Val Asn His Glu Glu Leu Glu Arg  
 785 790 795 800  
 Ser Pro Ser Gly Phe Ser Ile Ser Gln Asp Arg Asp Asp Tyr Val Phe  
 805 810 815  
 Gly Pro Ser Gly Gly Ser Ser Trp Met Gly Glu Lys Arg Tyr Leu Ala  
 820 825 830

52

Glu Gly Glu Thr Asp Thr Asp Thr Asp Pro Phe Thr Pro Ser Gly Ser  
835 840 845

Met Pro Met Ser Ser Thr Gly Asp Gly Ile Ser Asp Ser Ile Trp Thr  
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Pro Ser Asn Lys Pro Ile  
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<212> DNA  
<213> Homo sapiens

<220>  
<221> mutation  
<222> (118)  
<223> This nucleotide is deleted in a person with JME.

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gtcctggaag 130

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<400> 93  
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agg	cacc	atg	gtg	cag	aag	tgc	cgc	aac	ggc	ggc	gta	tac	ccc	ggc	ccg				169
		Met	Val	Gln	Lys	Ser	Arg	Asn	Gly	Gly	Val	Tyr	Pro	Gly	Pro				
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agc	ggg	gag	aag	aag	ctg	aag	gtg	ggc	ttc	gtg	ggg	ctg	gac	ccc	ggc				217
Ser	Gly	Glu	Lys	Lys	Leu	Lys	Val	Gly	Phe	Val	Gly	Leu	Asp	Pro	Gly				
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gcg	ccc	gac	tcc	acc	cgg	gac	ggg	gcg	ctg	ctg	atc	gcc	ggc	tcc	gag				265
Ala	Pro	Asp	Ser	Thr	Arg	Asp	Gly	Ala	Leu	Leu	Ile	Ala	Gly	Ser	Glu				
				35					40					45					
gcc	ccc	aag	cgc	ggc	agc	atc	ctc	agc	aaa	cct	cgc	gcg	ggc	ggc	gcg				313
Ala	Pro	Lys	Arg	Gly	Ser	Ile	Leu	Ser	Lys	Pro	Arg	Ala	Gly	Gly	Ala				
			50					55					60						
ggc	gcc	ggg	aag	ccc	ccc	aag	cgc	aac	gcc	ttc	tac	cgc	aag	ctg	cag				361
Gly	Ala	Gly	Lys	Pro	Pro	Lys	Arg	Asn	Ala	Phe	Tyr	Arg	Lys	Leu	Gln				
		65					70					75							
aat	ttc	ctc	tac	aac	gtg	ctg	gag	cgg	ccg	cgc	ggc	tgg	gcg	ttc	atc				409
Asn	Phe	Leu	Tyr	Asn	Val	Leu	Glu	Arg	Pro	Arg	Gly	Trp	Ala	Phe	Ile				
	80					85					90								
tac	cac	gcc	tac	gtg	ttc	ctc	ctg	gtt	ttc	tcc	tgc	ctc	gtg	ctg	tct				457
Tyr	His	Ala	Tyr	Val	Phe	Leu	Leu	Val	Phe	Ser	Cys	Leu	Val	Leu	Ser				
	95				100					105					110				
gtg	ttt	tcc	acc	atc	aag	gag	tat	gag	aag	agc	tgc	gag	ggg	gcc	ctc				505
Val	Phe	Ser	Thr	Ile	Lys	Glu	Tyr	Glu	Lys	Ser	Ser	Glu	Gly	Ala	Leu				
				115					120					125					
tac	atc	ctg	gaa	atc	gtg	act	atc	gtg	gtg	ttt	ggc	gtg	gag	tac	ttc				553
Tyr	Ile	Leu	Glu	Ile	Val	Thr	Ile	Val	Val	Phe	Gly	Val	Glu	Tyr	Phe				
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gtg	cgg	atc	tgg	gcc	gca	ggc	tgc	tgc	tgc	cgg	tac	cgt	ggc	tgg	agg				601
Val	Arg	Ile	Trp	Ala	Ala	Gly	Cys	Cys	Cys	Arg	Tyr	Arg	Gly	Trp	Arg				
		145					150					155							
ggg	cgg	ctc	aag	ttt	gcc	cgg	aaa	ccg	ttc	tgt	gtg	att	gac	atc	atg				649
Gly	Arg	Leu	Lys	Phe	Ala	Arg	Lys	Pro	Phe	Cys	Val	Ile	Asp	Ile	Met				
	160					165					170				</				



54

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Ser Val Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile	
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Gly Phe Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu	
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Lys Gly Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp	
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Gly Leu Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln	
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Thr Trp Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val	
290 295 300	
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Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu	
305 310 315	
aag gtt cag gag cag cac agg cag aag cac ttt gag aag agg cgg aac	1129
Lys Val Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn	
320 325 330	
ccg gca gca ggc ctg atc cag tcg gcc tgg aga ttc tac gcc acc aac	1177
Pro Ala Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn	
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Leu Ser Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr	
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Val Thr Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser	
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Arg Leu Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys	
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Ser Lys Ser Gly Leu Ala Phe Arg Lys Asp Pro Pro Pro Glu Pro Ser	
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ctc ggg aag gtg gag aag cag gtc ttg tcc atg gag aag aag ctg gac	2041
Leu Gly Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu Asp	
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Phe Leu Val Asn Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu	
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acc gag gcc tac ttt ggg gcc aaa gag ccg gag ccg gcg ccg ccg tac	2137
Thr Glu Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr	
655 660 665 670	
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His Ser Pro Glu Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile	
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Val Lys Ile Val Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser	
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Gln Pro Gln Ser His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly	
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Asp His Gly Ser Leu Val Arg Ile Pro Pro Pro Pro Ala His Glu Arg	
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770 775 780	
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785 790 795	
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Asp Ala Leu Asn Ser Cys Tyr Ala Ala Val Ala Pro Cys Ala Lys Val	
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Arg Pro Tyr Ile Ala Glu Gly Glu Ser Asp Thr Asp Ser Asp Leu Cys	
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865 870 875

ggc cag tgg acc cgc ccg cgg ccc tcc tca gca cgg tgc ctc cga ggt 2809  
Gly Gln Trp Thr Arg Pro Arg Pro Ser Ser Ala Arg Cys Leu Arg Gly  
880 885 890

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Phe	Glu	Ala	Gly	Thr	Leu	Trp	Gly	Pro	Phe	Leu	Thr	Val	Thr	Glu	Cys	
895					900					905					910	

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915 920 925

ggc tcc tgg cag tgacctttta caaaagttaa tttccaacag gggctggagg 2957  
Gly Ser Trp Gln  
930

gctgggcagg gcctgtggct ccaggagcag cgtgcaggag caaggctgcc ctgtccactc 3017

tgctcaagqc cgcggccgac atcagcccgg tgtgaagagg ggcggagtga tgacgggtgt 3077

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<400> 96

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Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro  
35 40 45

Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala  
50 55 60

58

Gly	Lys	Pro	Pro	Lys	Arg	Asn	Ala	Phe	Tyr	Arg	Lys	Leu	Gln	Asn	Phe	65	70	75	80
Leu	Tyr	Asn	Val	Leu	Glu	Arg	Pro	Arg	Gly	Trp	Ala	Phe	Ile	Tyr	His	85	90	95	
Ala	Tyr	Val	Phe	Leu	Leu	Val	Phe	Ser	Cys	Leu	Val	Leu	Ser	Val	Phe	100	105	110	
Ser	Thr	Ile	Lys	Glu	Tyr	Glu	Lys	Ser	Ser	Glu	Gly	Ala	Leu	Tyr	Ile	115	120	125	
Leu	Glu	Ile	Val	Thr	Ile	Val	Val	Phe	Gly	Val	Glu	Tyr	Phe	Val	Arg	130	135	140	
Ile	Trp	Ala	Ala	Gly	Cys	Cys	Cys	Arg	Tyr	Arg	Gly	Trp	Arg	Gly	Arg	145	150	155	160
Leu	Lys	Phe	Ala	Arg	Lys	Pro	Phe	Cys	Val	Ile	Asp	Ile	Met	Val	Leu	165	170	175	
Ile	Ala	Ser	Ile	Ala	Val	Leu	Ala	Ala	Gly	Ser	Gln	Gly	Asn	Val	Phe	180	185	190	
Ala	Thr	Ser	Ala	Leu	Arg	Ser	Leu	Arg	Phe	Leu	Gln	Ile	Leu	Arg	Met	195	200	205	
Ile	Arg	Met	Asp	Arg	Arg	Gly	Gly	Thr	Trp	Lys	Leu	Leu	Gly	Ser	Val	210	215	220	
Val	Tyr	Ala	His	Ser	Lys	Glu	Leu	Val	Thr	Ala	Trp	Tyr	Ile	Gly	Phe	225	230	235	240
Leu	Cys	Leu	Ile	Leu	Ala	Ser	Phe	Leu	Val	Tyr	Leu	Ala	Glu	Lys	Gly	245	250	255	
Glu	Asn	Asp	His	Phe	Asp	Thr	Tyr	Ala	Asp	Ala	Leu	Trp	Trp	Gly	Leu	260	265	270	
Ile	Thr	Leu	Thr	Thr	Ile	Gly	Tyr	Gly	Asp	Lys	Tyr	Pro	Gln	Thr	Trp	275	280	285	
Asn	Gly	Arg	Leu	Leu	Ala	Ala	Thr	Phe	Thr	Leu	Ile	Gly	Val	Ser	Phe	290	295	300	
Phe	Ala	Leu	Pro	Ala	Gly	Ile	Leu	Gly	Ser	Gly	Phe	Ala	Leu	Lys	Val	305	310	315	320
Gln	Glu	Gln	His	Arg	Gln	Lys	His	Phe	Glu	Lys	Arg	Arg	Asn	Pro	Ala	325	330	335	
Ala	Gly	Leu	Ile	Gln	Ser	Ala	Trp	Arg	Phe	Tyr	Ala	Thr	Asn	Leu	Ser	340	345	350	

Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr Val Thr  
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 Arg Ser Pro Ser Ala Asp Gln Ser Leu Glu Asp Ser Pro Ser Lys Val  
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 Val Met Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu Ser Leu Arg  
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 Val Gly Arg Gly Pro Ala Ile Thr Asp Lys Asp Arg Thr Lys Gly Pro  
 595 600 605  
 Ala Glu Ala Glu Leu Pro Glu Asp Pro Ser Met Met Gly Arg Leu Gly  
 610 615 620  
 Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu Asp Phe Leu  
 625 630 635 640

60

Val Asn Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu Thr Glu  
 645 650 655  
 Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr His Ser  
 660 665 670  
 Pro Glu Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile Val Lys  
 675 680 685  
 Ile Val Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser Ala Pro  
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 Gln Ser His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly Asp His  
 725 730 735  
 Gly Ser Leu Val Arg Ile Pro Pro Pro Pro Ala His Glu Arg Ser Leu  
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 Ser Ala Tyr Gly Gly Gly Asn Arg Ala Ser Met Glu Phe Leu Arg Gln  
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 Glu Asp Thr Pro Gly Cys Arg Pro Pro Glu Gly Asn Leu Arg Asp Ser  
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 Asp Thr Ser Ile Ser Ile Pro Ser Val Asp His Glu Glu Leu Glu Arg  
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 Ser Phe Ser Gly Phe Ser Ile Ser Gln Ser Lys Glu Asn Leu Asp Ala  
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 Cys Gly Pro Pro Pro Arg Ser Ala Thr Gly Glu Gly Pro Phe Gly Asp  
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 Val Gly Trp Ala Gly Pro Gly Pro Gly Ser Glu Ala Ala Leu Gly Gln  
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 Trp Thr Arg Pro Arg Pro Ser Ser Ala Arg Cys Leu Arg Gly Phe Glu  
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 Ala Gly Thr Leu Trp Gly Pro Phe Leu Thr Val Thr Glu Cys Gly Gly  
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<210> 104

<211> 241

<212> DNA

<213> Homo sapiens

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&lt;210&gt; 106

&lt;211&gt; 232

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 106

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&lt;210&gt; 107

&lt;211&gt; 128

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 107

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&lt;210&gt; 108

&lt;211&gt; 318

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 108

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&lt;210&gt; 113

&lt;211&gt; 236

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 113

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&lt;210&gt; 114

&lt;211&gt; 636

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 114

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68

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&lt;210&gt; 119

&lt;211&gt; 973

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 119

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&lt;210&gt; 120

&lt;211&gt; 1117

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 120

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&lt;210&gt; 121

&lt;211&gt; 1083

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 121

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&lt;213&gt; Homo sapiens

&lt;400&gt; 122

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/22375

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1, 325; 530/387.1; 536/23.1, 24.3, 24.31; 800/3, 13, 14, 18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	STOFFEL et al. Epilepsy genes: excitement traced to potassium channels. Nature Genetics. January 1998, Vol. 18, No. 1, pages 6-8, entire document.	1-70
Y, P	SINGH et al. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nature Genetics. January 1998, Vol. 18, No. 1, pages 25-29, entire document.	1-70
Y, P	CHARLIER et al. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nature Genetics. January 1998, Vol. 18, No. 1, pages 53-55, entire document.	1-70

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search

06 DECEMBER 1998

Date of mailing of the international search report

22 JAN 1999

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/22375

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEWIS et al. Localization of a gene for a glutamate binding subunit of a NMDA receptor (GRINA) to 8q24. Genomics. 1996, Vol. 32, No. 1, pages 131-133, entire document.	1-70
Y	STEINLEIN et al. Exon-intron structure of the human neuronal nicotinic acetylcholine receptor alpha4 subunit (CHRNA4). Genomics. 1996, Vol. 32, No. 1, pages 289-294, entire document.	1-70
Y	STEINLEIN.O. Detection of a CfoI polymorphism within exon 5 of the human neuronal nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4). Hum. Genet. 1995, Vol. 96, page 130, entire document.	1-70
Y	STEINLEIN et al. Benign familial neonatal convulsions: confirmation of genetic heterogeneity and further evidence for a second locus on chromosome 8q. Hum. Genet. 1995, Vol. 95, pages 411-415, entire document.	1-70

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/22375

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 1, 2, 4-14, 16-19, 21-24, 26, 28, 32, 33, 35-37, 40, 42, 44, 48, 51, 53, 55, 57, 59, 63, 65-70  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
Please See Extra Sheet.
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/22375

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 21/04; C07K 16/00; C12N 15/00, 15/11, 15/63, 15/85, 15/86

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/320.1, 325; 530/387.1; 536/23.1, 24.3, 24.31; 800/3, 13, 14, 18

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS

STN (file: medicine)

search terms: juvenile myoclonic epilepsy, JME, rolandic epilepsy, benign familial neonatal convulsions, BFNC, KCNQ2, KCNQ3, KVEBN1, KVEBN2

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

Insofar as the claims recite SEQ ID NO.'s or depend from claims that recite SEQ ID NO.'s, the claims were found to be unsearchable, because the computer readable format (CRF) was found to be technically bad. Thus, the claims were searched based on the description provided in the disclosure and keywords from the claims to the best of the ability of the examiner.





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C07H 21/04, C07K 16/00, C12N 15/00, 15/11, 15/63, 15/85, 15/86</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/21875</b> <b>(43) International Publication Date:</b> 6 May 1999 (06.05.99)
<b>(21) International Application Number:</b> PCT/US98/22375 <b>(22) International Filing Date:</b> 23 October 1998 (23.10.98)  <b>(30) Priority Data:</b> 60/063,147                      24 October 1997 (24.10.97)                      US  <b>(71) Applicant:</b> UNIVERSITY OF UTAH RESEARCH FOUNDATION [US/US]; Suite 170, 421 Wakara Way, Salt Lake City, UT 84108 (US).  <b>(72) Inventors:</b> SINGH, Nanda, A.; Timberlakes #101, Heber City, UT 84032 (US). LEPPERT, Mark, F.; 1466 Westminster Avenue, Salt Lake City, UT 84105 (US). CHARLIER, Carole; 7, rue de Blindel, B-4141 Sprimont (BE).  <b>(74) Agents:</b> IHNEN, Jeffrey, L. et al.; Rothwell, Figg, Ernst & Kurz, Columbia Square, Suite 701 East, 555 13th Street N.W., Washington, DC 20004 (US).		<b>(81) Designated States:</b> CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> KCNQ2 AND KCNQ3-POTASSIUM CHANNEL GENES WHICH ARE MUTATED IN BENIGN FAMILIAL NEONATAL CONVULSIONS (BFNC) AND OTHER EPILEPSIES  <b>(57) Abstract</b> <p>Generalized idiopathic epilepsies (IGE) cause 40 % of all seizures and commonly have a genetic basis. One type of IGE is Benign Familial Neonatal Convulsions (BFNC), a dominantly inherited disorder of newborns. A submicroscopic deletion of chromosome 20q13.3 which cosegregates with seizures in BFNC family has been identified. Characterization of cDNAs spanning the deleted region identified a novel voltage-gated potassium channel, KCNQ2, which belongs to a new KCNQ1-like class of potassium channels. Nine other BFNC probands were shown to have <i>KCNQ2</i> mutations including three missense mutations, three frameshifts, two nonsense mutations, and one splice site mutation. A second gene, <i>KCNQ3</i>, was found in a separate BFNC family in which the mutation had been localized to chromosome 8. A missense mutation was found in this gene in perfect cosegregation with the BFNC phenotype in this latter family. This demonstrates that defects in potassium channels can cause epilepsy. Furthermore, some members of one of the BFNC families with a mutation in <i>KCNQ2</i> also exhibited rolandic epilepsy and one individual with juvenile myoclonic epilepsy has a mutation in an alternative exon of <i>KCNQ3</i>.</p>		

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DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TITLE OF THE INVENTION

KCNQ2 and KCNQ3 - POTASSIUM CHANNEL GENES WHICH ARE MUTATED IN BENIGN FAMILIAL NEONATAL CONVULSIONS (BFNC) AND OTHER EPILEPSIES

This application was made with Government support under Grant Nos. R01-NS32666  
5 funded by the National Institutes of Health, Bethesda, Maryland.

CROSS REFERENCE TO RELATED APPLICATIONS

This application is related to Serial No. 60/063,147, filed 24 October 1997, to which priority is claimed and which is incorporated herein by reference.

10

BACKGROUND OF THE INVENTION

Epileptic disorders affect about 20 to 40 million people worldwide. Generalized idiopathic epilepsies (IGE) cause 40% of all epileptic disorders and commonly have a genetic basis (Plouin, 1994). Most of the IGEs that are inherited are complex, non-monogenic diseases. One type of IGE  
15 is Benign Familial Neonatal Convulsions (BFNC), a dominantly inherited disorder of newborns (Ronen et al., 1993; Hauser and Kurland, 1975). BFNC (OMIM 121200) is an autosomal dominantly inherited epilepsy of the newborn infant. This idiopathic, generalized epilepsy typically has an onset of seizures on day two to four of life. Spontaneous remission of the seizures occurs between two to fifteen weeks (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975).  
20 Seizures typically start with a tonic posture, ocular symptoms and other autonomic features which then often progress to clonic movements and motor automatisms. These neonates thrive normally between the seizures, and their neurologic examinations and later development indicate normal brain functioning (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975). However, in spite of normal neurologic development, seizures recur later in life in approximately 16% of BFNC cases  
25 compared with a 2% cumulative lifetime risk of epilepsy in the general population (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975).

Genetic heterogeneity of BFNC has been observed (Ryan et al., 1991). Two loci, *EBN1* and *EBN2*, have been mapped by linkage analysis to chromosome 20q13 (Leppert et al., 1989; Malafosse et al., 1992) and chromosome 8q24 (Lewis et al., 1993; Steinlein et al., 1995),  
30 respectively.

The nomenclature of the genes of this invention as well as related genes has changed over time. Two of the genes of this invention from humans are now referred to as *KCNQ2* and *KCNQ3*.

These had originally been named *KVEBN1* and *KVEBN2*, respectively. The two sets of names are equivalent and can be used interchangeably, but the accepted nomenclature is now *KCNQ2* and *KCNQ3* and these names will be used herein. Also, the related gene *KCNQ1* had originally been called *KVLQT1* in the literature, but again the accepted name now is *KCNQ1* and this name will be used herein.

Linkage analysis in a large kindred demonstrated that a gene, herein called *KCNQ2*, responsible for BFNC maps to chromosome 20q13.3 close to the markers D20S20 and D20S19 (Leppert et al., 1989). Following the initial report, two centers confirmed linkage of BFNC to the same two genetic markers on chromosome 20, termed the EBN1 (epilepsy benign neonatal type 1) locus (Ryan et al., 1991; Malafosse et al., 1992; Steinlein et al., 1992). A more distal marker, D20S24, shows complete co-segregation with the BFNC phenotype in chromosome 20 linked families. Finding a distal flanking marker for the BFNC locus has not been successful probably because of its proximity to the telomere. This telomeric region is characterized by a high recombination rate between markers when compared to the physical distance (Steinlein et al., 1992). In fact, Steinlein et al. have demonstrated that the three markers D20S19, D20S20 and D20S24 are contained on the same 450 Mb Mlu I restriction fragment (Steinlein et al., 1992). All of the families in the present study used to find and study *KCNQ2* show linkage to chromosome 20q markers with LOD scores of greater than 3.0 or have probands with clinical manifestations consistent with BFNC (Leppert et al., 1993). Each subject and control signed a Consent for Participation in these studies approved by the Institutional Review Board for Human Subject Research at their home institution. To find a gene responsible for BFNC, we narrowed a BFNC region with a sub-microscopic deletion in a single family, identified candidate cDNAs in this deletion, and then searched for mutations in other BFNC families. The gene has been identified and sequenced. Several distinct mutations have been found in this gene. These include a large deletion, three missense mutations, three frameshift mutations, two nonsense mutations and one splice site mutation. One of these mutations is associated with rolandic epilepsy as described in the Examples below.

A second chromosomal locus, EBN2, has also been identified for BFNC. Lewis et al. (1993) demonstrated linkage to markers on chromosome 8q24 in a single Hispanic family affected with BFNC. Evidence for this second locus was also reported in a Caucasian pedigree (Steinlein et al., 1995). The gene, herein called *KCNQ3*, responsible for EBN2 was mapped to chromosome 8, between markers D8S256 and D8S284 on a radiation hybrid map (Lewis et al., 1995). *KCNQ3* has been identified as set out in the examples of the instant disclosure. *KCNQ3* was screened for



mutations in the large BFNC family previously linked to chromosome 8q24 in the same marker interval (Ryan et al., 1991; Lewis et al., 1993). A missense mutation was found in the critical pore region in perfect cosegregation with the BFNC phenotype. The same conserved amino acid is also mutated in *KCNQ1* in an LQT patient (Wang et al., 1996). Furthermore, the segment of mouse chromosome 15 that harbors the *stargazer* (*stg*) locus (Noebels et al., 1990; Letts et al., 1997) is homologous to the human 8q24 region and the *stg* phenotype is close to a common form of IGE, the absence epilepsy. *KCNQ2*, *KCNQ3* and other undiscovered genes of the same family of K<sup>+</sup> channels are strong candidates for other, more common IGEs. One individual with juvenile myoclonic epilepsy has been found who has a mutation in an alternative exon of *KCNQ3* as shown in the Examples below.

IGEs include many different types of seizures. Common IGEs include generalized tonic-clonic seizure (GTCS), absence epilepsy of childhood (AEC), juvenile absence epilepsy (JAE) and juvenile myoclonic epilepsy (JME). Reutens and Berkovic (1995) have shown that the boundaries between the different IGE syndromes are indistinct and suggest that neurobiological and possibly genetic relationships exist between these syndromes. Interestingly, using non-parametric linkage methods, Zara et al. (1995) obtained evidence for involvement of an epilepsy locus at chromosome 8q24 in a panel of families with multiple cases of IGEs. Furthermore, in a population study, Steinlein et al. (1997) recently described a weak allelic association at the *CHRNA4* locus, on chromosome 20q13.3, physically close to *KCNQ2*, in a group of unrelated patients with multiple forms of IGEs. Finally, an epileptic mutant mouse *stargazer* (*stg*) (Noebels et al., 1990) is a genetic model of spike wave epilepsy. This is a recessive mutation and the phenotype is related to a common form of human IGE, the absence epilepsy. *Stg* has been mapped on mouse chromosome 15 in a region homologous to the human 8q24 region. Screening the mouse homolog of *KCNQ3* for mutations in an affected mouse will assess the hypothesis that the same gene is responsible for both BFNC and *Stargazer* phenotypes.

The present invention is directed to both *KCNQ2* and *KCNQ3* and their gene products, mutations in the genes, the mutated genes, probes for the wild-type and mutated genes, and to a process for the diagnosis and prevention of BFNC. Each of the genes encodes a potassium channel protein. The instant work shows that some families with BFNC have mutations in either *KCNQ2* or *KCNQ3*. BFNC is diagnosed in accordance with the present invention by analyzing the DNA sequence of the *KCNQ2* and/or *KCNQ3* gene of an individual to be tested and comparing the respective DNA sequence to the known DNA sequence of a normal *KCNQ2* and/or *KCNQ3* gene.

Alternatively, the *KCNQ2* gene and/or *KCNQ3* gene of an individual to be tested can be screened for mutations which cause BFNC. Prediction of BFNC will enable practitioners to prevent this disorder using existing medical therapy. Furthermore, a mutation in *KCNQ2* has been found which is associated with rolandic epilepsy and a mutation in *KCNQ3* has been found which is associated with JME. These two forms of epilepsy may also be diagnosed in accord with the invention.

Mouse genes homologous to the human *KCNQ2* and *KCNQ3* have also been found and sequenced and the sequences are disclosed. The mouse *KCNQ2* gene has been only partially isolated and sequenced (shown as SEQ ID NO:88), the 3' end not yet having been found. The complete mouse *KCNQ3* gene has been isolated and sequenced (shown as SEQ ID NO:90).

The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference, and for convenience are respectively grouped in the appended List of References.

#### SUMMARY OF THE INVENTION

The present invention demonstrates a molecular basis of Benign Familial Neonatal Convulsions (BFNC) as well as for rolandic epilepsy and juvenile myoclonic epilepsy. More specifically, the present invention has determined that molecular variants of either the *KCNQ2* gene or *KCNQ3* gene cause or are involved in the pathogenesis of these three forms of epilepsy. Genotypic analyses show that *KCNQ2* is linked to BFNC in ten unrelated families and *KCNQ3* is linked to BFNC in one other family. Furthermore, one mutation in the *KCNQ2* gene in two individuals of one family has been associated with rolandic epilepsy and one individual with a mutation in *KCNQ3* has been diagnosed with juvenile myoclonic epilepsy. Analysis of the *KCNQ2* and *KCNQ3* genes will provide an early diagnosis of subjects with BFNC, rolandic epilepsy or JME. The diagnostic method comprises analyzing the DNA sequence of the *KCNQ2* and/or the *KCNQ3* gene of an individual to be tested, and comparing it with the DNA sequence of the native, non-variant gene. In a second embodiment, the *KCNQ2* and/or *KCNQ3* gene of an individual to be tested is screened for mutations which cause BFNC, rolandic epilepsy or JME. The ability to predict these epilepsies will enable physicians to prevent the disease with medical therapy such as drugs which directly or indirectly modulate K<sup>+</sup> ion channels.

The invention shows that various genetic defects of a potassium channel are responsible for the human idiopathic epilepsy of BFNC, rolandic epilepsy and/or JME. This finding adds to the growing list of channelopathies in humans (Ptacek, 1997). Importantly, this result suggests that

drugs which directly or indirectly modulate K<sup>+</sup> ion channels will be helpful in the treatment of seizure disorders.

### BRIEF DESCRIPTION OF THE FIGURES

5        The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1. Southern blot of kindred 1547 (showing 4 generations listed as I, II, III and IV) genomic DNA cut with *TaqI* and probed with the VNTR marker D20S24 showing a null allele in 10 affected individuals. Line A shows genotype misinheritances shown in boxes; line B shows corrected genotypes. The "N" indicates non-penetrant individuals.

Figures 2A-C. Metaphase spreads of cell lines from affected individuals of kindred 1547 probed with P1-KO9-7 (Figure 2C) and P1-KO9-6b (Figure 2B) genomic P1 clones and the 12 kb D20S24 RFLP marker (Figure 2A) demonstrating a deletion of D20S24.

15        Figure 3. Amino acid alignment between human members (KCNQ2, KCNQ3 and KCNQ1) and the *C. elegans* homologue (nKQT1) of the KQT-like family. The six transmembrane domains and the pore are indicated by a solid line located above the corresponding sequence. The conserved charged amino acids in the transmembrane domains are highlighted in gray. The sequence of KCNQ2 is SEQ ID NO:2, the sequence of KCNQ3 is SEQ ID NO:7, the sequence of nKQT1 is SEQ 20 ID NO:3 and the sequence of KCNQ1 is SEQ ID NO:4.

Figure 4. Figure 4 shows a three generation pedigree with BFNC linked to chromosome 20. BFNC individuals are depicted by filled in black circles and squares. The data is from kindred 1504 which shows variants in the KCNQ2 pores. The lower portion of the figure shows the cosegregation of the variant form which is present only in affected individuals. Sequence analysis revealed the 25 existence of a two base pair insertion in affected individuals showing the upper two (variant) bands.

Figure 5. Radiation Hybrid Mapping of the *KCNQ3* locus. Interpair LOD scores are given above the center line and distance between marker pairs, in cR<sub>5000</sub>, is shown below. The odds against inversion for adjacent loci is also given for each marker pair.

Figure 6. Figure 6 shows a three generation pedigree with BFNC linked to chromosome 8. 30 BFNC individuals are depicted by filled in black circles and squares. The non-penetrant individual III-8 is indicated by the symbol NP. The lower portion of the figure shows the co-segregation of the 187 bp SSCP variant, present only in affected and non-penetrant individuals (arrow).

Figures 7A-O. Intron/exon sequence is shown for *KCNQ2*. Exon sequence is shown in bold and primer sequence is in italics. The primer sequences are found in Table 4. The sequences are SEQ ID NOs:100-114.

Figures 8A-O. Intron/exon sequence is shown for *KCNQ3*. Exon sequence is shown uppercase and intron is shown lowercase and primer sequences are underlined. The primer sequences are found in Table 5. The sequences are SEQ ID NOs:115-129. Figure 8I shows the alternatively spliced exon found in a JME patient. Figure 8N shows an "N" in the 3' intron region. This "N" stands for Alu repeats which are found in this region.

#### 10 BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 is the cDNA sequence for *KCNQ2*.

SEQ ID NO:2 is the amino acid sequence for *KCNQ2*.

SEQ ID NO:3 is the amino acid sequence for nKQT1.

SEQ ID NO:4 is the amino acid sequence for *KCNQ1*.

15 SEQ ID NO:5 is nucleotide sequence at the intron/exon junction of the 3' end of the intron interrupting the two exons which encode amino acid 544 of *KCNQ2*.

SEQ ID NO:6 is the cDNA sequence for *KCNQ3*.

SEQ ID NO:7 is the amino acid sequence for *KCNQ3*.

SEQ ID NOs:8-9 are primers used for somatic cell hybrid panel genotyping (Example 7).

20 SEQ ID NOs:10-11 are primers used for genotyping a chromosome 8 radiation hybrid panel (Example 8).

SEQ ID NOs:12-17 are primers used to perform RACE to obtain full length cDNA (Example 9).

SEQ ID NOs:18-19 are primers used to prepare a PCR fragment which identified an SSCP variant for *KCNQ3*.

25 SEQ ID NOs:20-21 are hypothetical nucleic acid sequences to demonstrate calculation of percent homology between two nucleic acids.

SEQ ID NOs:22-53 are primers for amplifying portions of *KCNQ2*.

SEQ ID NOs:54-87 are primers for amplifying portions of *KCNQ3*.

SEQ ID NO:88 is a partial mouse *KCNQ2*.

30 SEQ ID NO:89 is a partial mouse *KCNQ2* encoded by SEQ ID NO:88.

SEQ ID NO:90 is a mouse *KCNQ3*.

SEQ ID NO:91 is the mouse *KCNQ3* encoded by SEQ ID NO:90.

SEQ ID NO:92 is an alternative exon found in *KCNQ3*.

SEQ ID NOs:93-94 are primers based on mouse sequence to amplify 5' end of human *KCNQ3*.

SEQ ID NO:95 is a mutated human *KCNQ2* with a GGGCC insertion after nucleotide 2736.

SEQ ID NO:96 is a mutated human *KCNQ2* encoded by SEQ ID NO:95.

5 SEQ ID NOs:97-99 are primers for amplifying portions of *KCNQ2*.

SEQ ID NOs:100-114 are intron/exon sequence for *KCNQ2* (Figures 7A-O).

SEQ ID NOs:115-129 are intron/exon sequence for *KCNQ3* (Figures 8A-O).

### DETAILED DESCRIPTION OF THE INVENTION

10 The present invention is directed to the determination that BFNC maps to the *KCNQ2* gene and to the *KCNQ3* gene and that molecular variants of these genes cause or are involved in the pathogenesis of BFNC, rolandic epilepsy and/or JME. More specifically, the present invention relates to mutations in the *KCNQ2* gene and in the *KCNQ3* gene and their use in the diagnosis of BFNC, rolandic epilepsy and JME. The present invention is further directed to methods of  
15 screening humans for the presence of *KCNQ2* and/or *KCNQ3* gene variants which cause BFNC, rolandic epilepsy and/or JME. Since these forms of epilepsy can now be detected earlier (i.e., before symptoms appear) and more definitively, better treatment options will be available in those individuals identified as having BFNC, rolandic epilepsy or JME. The present invention is also directed to methods for screening for drugs useful in treating or preventing BFNC, rolandic epilepsy  
20 or JME.

The present invention provides methods of screening the *KCNQ2* and/or *KCNQ3* gene to identify mutations. Such methods may further comprise the step of amplifying a portion of the *KCNQ2* or *KCNQ3* gene, and may further include a step of providing a set of polynucleotides which are primers for amplification of said portion of the *KCNQ2* or *KCNQ3* gene. The method is useful  
25 for identifying mutations for use in either diagnosis of or prognosis of BFNC, rolandic epilepsy and JME.

Benign Familial Neonatal Convulsion is an autosomal dominantly inherited disorder that causes epilepsy of the newborn infant. This idiopathic, generalized epilepsy typically has an onset of seizures on day two to four of life. Spontaneous remission of the seizures occurs between two  
30 to fifteen weeks (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975). Seizures typically start with a tonic posture, ocular symptoms and other autonomic features which then often progress to clonic movements and motor automatisms. These neonates thrive normally between the seizures,

and their neurologic examinations and later development indicate normal brain functioning (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975). However, in spite of normal neurologic development, seizures recur later in life in approximately 16% of BFNC cases compared with a 2% cumulative lifetime risk of epilepsy in the general population (Ronen et al., 1993; Plouin, 1994; 5 Hauser and Kurland, 1975).

Linkage analysis in a large kindred demonstrated that a gene responsible for BFNC maps to chromosome 20q13.3 close to the markers D20S20 and D20S19 (Leppert et al, 1989). Following the initial report, two centers confirmed linkage of BFNC to the same two genetic markers on chromosome 20, termed the EBN1 (epilepsy benign neonatal type 1) locus (Ryan et al., 1991; 10 Malafosse et al., 1992). A more distal marker, D20S24, shows complete co-segregation with the BFNC phenotype in chromosome 20 linked families. Finding a distal flanking marker for the BFNC locus has not been successful probably because of its proximity to the telomere. This telomeric region is characterized by a high recombination rate between markers when compared to the physical distance (Steinlein et al., 1992). In fact, Steinlein et al. have demonstrated that the three 15 markers D20S19, D20S20 and D20S24 are contained on the same 450 Mb Mlu I restriction fragment (Steinlein et al., 1992). All of the families in the present study for *KCNQ2* show linkage to chromosome 20q markers with LOD scores of greater than 3.0 or have probands with clinical manifestations consistent with BFNC (Leppert et al., 1993). To find this gene responsible for BFNC, we narrowed the BFNC region with a sub-microscopic deletion in a single family, identified 20 candidate cDNAs in this deletion, and then searched for mutations in other BFNC families.

A second chromosomal locus, EBN2, has also been identified for BFNC. Lewis et al. (1993) demonstrated linkage to markers on chromosome 8q24 in a single Hispanic family affected with BFNC. Evidence for this second locus was also reported in a Caucasian pedigree (Steinlein et al., 1995). The gene for EBN2, *KCNQ3*, has now been found and characterized as detailed in this 25 disclosure.

Finally, the present invention is directed to a method for screening drug candidates to identify drugs useful for treating or preventing BFNC, rolandic epilepsy or JME. Drug screening is performed by expressing mutant *KCNQ2* or mutant *KCNQ3* in cells, such as oocytes, mammalian cells or transgenic animals, and assaying the effect of a drug candidate on the *KCNQ2* or *KCNQ3* 30 potassium channel. The effect is compared to the *KCNQ2* or *KCNQ3* potassium channel activity obtained using the wild-type *KCNQ2* or *KCNQ3* gene.

Proof that the *KCNQ2* and *KCNQ3* genes are involved in causing BFNC, rolandic epilepsy and JME is obtained by finding sequences in DNA extracted from affected kindred members which create abnormal *KCNQ2* or abnormal *KCNQ3* gene products or abnormal levels of the gene products. Such susceptibility alleles will co-segregate with the disease in large kindreds. They will  
5 also be present at a much higher frequency in non-kindred individuals with epilepsy than in individuals in the general population. The key is to find mutations which are serious enough to cause obvious disruption to the normal function of the gene product. These mutations can take a number of forms. The most severe forms would be frame shift mutations or large deletions which would cause the gene to code for an abnormal protein or one which would significantly alter protein  
10 expression. Less severe disruptive mutations would include small in-frame deletions and nonconservative base pair substitutions which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic to an acidic amino acid or vice versa, from a hydrophobic to hydrophilic amino acid or vice versa, or other mutations which would affect secondary or tertiary protein structure. Silent mutations or those resulting in conservative  
15 amino acid substitutions would not generally be expected to disrupt protein function.

According to the diagnostic and prognostic method of the present invention, alteration of the wild-type *KCNQ2* or *KCNQ3* gene is detected. In addition, the method can be performed by detecting the wild-type *KCNQ2* or *KCNQ3* gene and confirming the lack of a cause of epilepsy as a result of this locus. "Alteration of a wild-type gene" encompasses all forms of mutations including  
20 deletions, insertions and point mutations in the coding and noncoding regions. Deletions may be of the entire gene or of only a portion of the gene. Point mutations may result in stop codons, frameshift mutations or amino acid substitutions. Somatic mutations are those which occur only in certain tissues and are not inherited in the germline. Germline mutations can be found in any of a body's tissues and are inherited. Point mutational events may occur in regulatory regions, such  
25 as in the promoter of the gene, leading to loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the *KCNQ2* or *KCNQ3* gene product, or to a decrease in mRNA stability or translation efficiency.

Useful diagnostic techniques include, but are not limited to fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded  
30 conformation analysis (SSCA), RNase protection assay, allele-specific oligonucleotide (ASO), dot blot analysis, hybridization using nucleic acid modified with gold nanoparticles and PCR-SSCP,

as discussed in detail further below. Also useful is the recently developed technique of DNA microchip technology.

The presence of BFNC, rolandic epilepsy or JME may be ascertained by testing any tissue of a human for mutations of the *KCNQ2* or *KCNQ3* gene. For example, a person who has inherited a germline *KCNQ2* mutation would be prone to develop BFNC. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the *KCNQ2* or *KCNQ3* gene. Alteration of a wild-type *KCNQ2* or *KCNQ3* allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing can detect sequence variation. Another approach is the single-stranded conformation polymorphism assay (SSCP) (Orita et al., 1989). This method does not detect all sequence changes, especially if the DNA fragment size is greater than 200 bp, but can be optimized to detect most DNA sequence variation. The reduced detection sensitivity is a disadvantage, but the increased throughput possible with SSCP makes it an attractive, viable alternative to direct sequencing for mutation detection on a research basis. The fragments which have shifted mobility on SSCP gels are then sequenced to determine the exact nature of the DNA sequence variation. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield et al., 1991), heteroduplex analysis (HA) (White et al., 1992) and chemical mismatch cleavage (CMC) (Grompe et al., 1989). None of the methods described above will detect large deletions, duplications or insertions, nor will they detect a regulatory mutation which affects transcription or translation of the protein. Other methods which might detect these classes of mutations such as a protein truncation assay or the asymmetric assay, detect only specific types of mutations and would not detect missense mutations. A review of currently available methods of detecting DNA sequence variation can be found in a recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific oligonucleotide (ASO) hybridization can be utilized to rapidly screen large numbers of other samples for that same mutation. Such a technique can utilize probes which are labeled with gold nanoparticles to yield a visual color result (Elghanian et al., 1997).



A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably with a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of BFNC, rolandic epilepsy or JME cases. Southern blots displaying hybridizing fragments differing in length from control DNA when probed with sequences near or including the *KCNQ2* locus indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis (PFGE) is employed.

Detection of point mutations may be accomplished by molecular cloning of the *KCNQ2* or *KCNQ3* allele and sequencing the allele using techniques well known in the art. Also, the gene or portions of the gene may be amplified, e.g., by PCR or other amplification technique, and the amplified gene or amplified portions of the gene may be sequenced.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis (SSCP) (Orita et al., 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell et al., 1990; Sheffield et al., 1989); 3) RNase protection assays (Finkelstein et al., 1990; Kinszler et al., 1991); 4) allele-specific oligonucleotides (ASOs) (Conner et al., 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and 6) allele-specific PCR (Ruano and Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular *KCNQ2* or *KCNQ3* mutation. If the particular mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

In the first three methods (SSCP, DGGE and RNase protection assay), a new electrophoretic band appears. SSCP detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a

denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

5 Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of samples. An example of a mismatch  
10 cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type *KCNQ2* or *KCNQ3* gene coding sequence. The riboprobe and either mRNA or DNA isolated from the person are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected  
15 by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the mRNA or gene, it will be  
20 desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., 1988; Shenk et al., 1975; Novack et al., 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA  
25 probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the *KCNQ2* or *KCNQ3* gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the *KCNQ2* or *KCNQ3* gene which have been amplified by use of PCR  
30 may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the gene sequence.

By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the gene. Hybridization of allele-specific probes with amplified *KCNQ2* or *KCNQ3* sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under high stringency hybridization conditions indicates  
5 the presence of the same mutation in the tissue as in the allele-specific probe. High stringency hybridization conditions are defined as those conditions which allow an 8 basepair stretch of a first nucleic acid (a probe) to bind to a 100% perfectly complementary 8 basepair stretch of nucleic acid while simultaneously preventing binding of said first nucleic acid to a nucleic acid which is not 100% complementary, i.e., binding will not occur if there is a mismatch.

10 The newly developed technique of nucleic acid analysis via microchip technology is also applicable to the present invention. In this technique, literally thousands of distinct oligonucleotide probes are built up in an array on a silicon chip. Nucleic acid to be analyzed is fluorescently labeled and hybridized to the probes on the chip. It is also possible to study nucleic acid-protein interactions using these nucleic acid microchips. Using this technique one can determine the presence of  
15 mutations or even sequence the nucleic acid being analyzed or one can measure expression levels of a gene of interest. The method is one of parallel processing of many, even thousands, of probes at once and can tremendously increase the rate of analysis. Several papers have been published which use this technique. Some of these are Hacia et al., 1996; Shoemaker et al., 1996; Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1996; Lipshutz et al., 1995. This method has already been  
20 used to screen people for mutations in the breast cancer gene BRCA1 (Hacia et al., 1996). This new technology has been reviewed in a news article in Chemical and Engineering News (Borman, 1996) and been the subject of an editorial (Nature Genetics, 1996). Also see Fodor (1997).

The most definitive test for mutations in a candidate locus is to directly compare genomic *KCNQ2* or *KCNQ3* sequences from patients with those from a control population. Alternatively,  
25 one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

Mutations from patients falling outside the coding region of *KCNQ2* or *KCNQ3* can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the genes. An early indication that mutations in noncoding regions are important may come  
30 from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in patients as compared to control individuals.

Alteration of *KCNQ2* or *KCNQ3* mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type gene. Alteration of wild-type genes can also be detected by screening for alteration of wild-type *KCNQ2* or *KCNQ3* protein. For example, monoclonal antibodies immunoreactive with *KCNQ2* or *KCNQ3* can be used to screen a tissue. Lack of cognate antigen would indicate a mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered *KCNQ2* or *KCNQ3* protein can be used to detect alteration of the wild-type *KCNQ2* or *KCNQ3* gene. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect *KCNQ2* or *KCNQ3* biochemical function. Finding a mutant *KCNQ2* or *KCNQ3* gene product indicates alteration of a wild-type *KCNQ2* or *KCNQ3* gene.

A mutant *KCNQ2* or *KCNQ3* gene or gene product can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant genes or gene products in tissues can be applied to other body samples. By screening such body samples, a simple early diagnosis can be achieved for BFNC, rolandic epilepsy or JME.

The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular *KCNQ2* or *KCNQ3* allele using PCR. The pairs of single-stranded DNA primers for *KCNQ2* or *KCNQ3* can be annealed to sequences within or surrounding the *KCNQ2* gene on chromosome 20 or *KCNQ3* gene on chromosome 8 in order to prime amplifying DNA synthesis of the gene itself. A complete set of these primers allows synthesis of all of the nucleotides of the gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele-specific primers can also be used. Such primers anneal only to particular *KCNQ2* or *KCNQ3* mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appended to their 5' ends. Thus, all nucleotides of the primers are derived from *KCNQ2* or *KCNQ3* sequence or sequences adjacent to *KCNQ2* or *KCNQ3*, except for the few nucleotides necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the

art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. Given the sequence of *KCNQ2* and *KCNQ3*, design of particular primers is well within the skill of the art.

The nucleic acid probes provided by the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase protection method for detecting point mutations already discussed above. The probes can be used to detect PCR amplification products. They may also be used to detect mismatches with the *KCNQ2* or *KCNQ3* gene or mRNA using other techniques.

It has been discovered that most individuals with the wild-type *KCNQ2* and *KCNQ3* genes do not have BFNC. However, mutations which interfere with the function of the *KCNQ2* or *KCNQ3* gene product are involved in the pathogenesis of BFNC. Thus, the presence of an altered (or a mutant) *KCNQ2* or *KCNQ3* gene which produces a protein having a loss of function, or altered function, directly causes BFNC which increases the risk of seizures. In order to detect a *KCNQ2* or *KCNQ3* gene mutation, a biological sample is prepared and analyzed for a difference between the sequence of the allele being analyzed and the sequence of the wild-type allele. Mutant *KCNQ2* or *KCNQ3* alleles can be initially identified by any of the techniques described above. The mutant alleles are then sequenced to identify the specific mutation of the particular mutant allele. Alternatively, mutant alleles can be initially identified by identifying mutant (altered) proteins, using conventional techniques. The mutant alleles are then sequenced to identify the specific mutation for each allele. The mutations, especially those which lead to an altered function of the protein, are then used for the diagnostic and prognostic methods of the present invention.

This is the first human idiopathic generalized epilepsy for which a  $K^+$  channel has been implicated. BFNC is considered to be a true idiopathic epilepsy without the degenerative characteristics associated with other syndromes such as progressive myoclonus epilepsy of the Unverricht-Lundborg type. It is not surprising, therefore, that an alteration in a gene which directly regulates neuronal excitability causes this epileptic disorder. Voltage-gated potassium channels repolarize neuronal membranes that have been depolarized by  $Na^+$  and  $Ca^{++}$  voltage-gated ion channels.  $K^+$  channels are also thought to repolarize neuronal membranes following activation of excitatory neurotransmitter ion channels, including glutamate and acetylcholine. In the presence of mutant *KCNQ2* or *KCNQ3* channels with reduced function, excitatory ligand and voltage-gated channels that are activated would remain open for a longer duration (Keating and Sanguinetti, 1996; Meldrum, 1995; McNamara, 1994). Such unchecked activity of excitatory systems could lead to

an epileptic phenotype. Electrophysiologic analysis of the mutant KCNQ2 and KCNQ3 channels will shed light on how the mutations identified in the current study produce an epileptic phenotype. It is likely that KCNQ2 and KCNQ3 will have biophysical properties similar to the delayed rectifier KCNQ1 channel. KCNQ1 alpha subunits coassemble with minK beta subunits to form  
5 heteromultimeric  $I_{Ks}$  channels in the heart (Sanguinetti et al., 1996). It is possible that KCNQ2 and KCNQ3 subunits coassemble with minK-like beta subunits in the brain. This interaction may also alter the gating properties of the resulting heteromultimeric channel as is the case for KCNQ1.

Mutations in  $K^+$  channels have been associated with epilepsy in only one other case, the weaver mouse, where a single missense mutation in the GIRK2 gene produces spontaneous seizures  
10 (Patil et al., 1995; Signorini et al., 1997). Mutations in  $K^+$  channels have been implicated in other human disorders such as the Long QT syndrome on chromosome 11 and ataxia/myokymia on chromosome 12 (Wang et al., 1996; Neyroud et al., 1997; Russell et al., 1996; Chandy and Gutman, 1995; Browne et al., 1994). Long QT is associated with four loci, two of which are the  $K^+$  channel genes HERG and KCNQ1. In KCNQ1, mutational hot spots have been identified in the pore and  
15 S6 domains where missense mutations in these regions account for a majority of the disease causing mutations in LQT (Russell et al., 1996; Wang et al., 1996).

Since the first publications of the finding of the *KCNQ2* and *KCNQ3* genes, there have been several more publications. Iannotti et al. (1998) found that there are two splice variants of *KCNQ2*. These are a long and a short form which differ in their C-termini. The long form is expressed  
20 exclusively in human brain (adult and fetal), where it is restricted to neuronal rather than glial cells. The short form is expressed weakly in adult brain but is prominent in fetal brain and testes (Iannotti et al., 1998). Gribkoff et al. (1998) cloned and expressed a mouse homologue of *KCNQ2* in *Xenopus* oocytes and performed two-electrode voltage clamp studies. Dworetzky et al. (1998) cloned a mouse homologue of *KCNQ2* and also noted alternative splice variants in the 3' region of  
25 the gene. They also performed Northern blots and measured polarization in *Xenopus* oocytes expressing the mouse gene. Yang et al. (1998) have also cloned and expressed the human *KCNQ2* and *KCNQ3*. They note that the encoded proteins act like KCNQ1 in eliciting voltage-gated, rapidly activating  $K^+$ -selective currents, but in contrast to KCNQ1, the KCNQ2 and KCNQ3 protein induced currents are not augmented by coexpression of KCNE1. However, coexpression of KCNQ2  
30 and KCNQ3 results in a substantial synergistic increase in current amplitude (Yang et al., 1998). Finally, Biervert et al. (1998) cloned human *KCNQ2* and expressed it in *Xenopus* oocytes.

### Definitions

The present invention employs the following definitions.

"**Amplification of Polynucleotides**" utilizes methods such as the polymerase chain reaction (PCR), ligation amplification (or ligase chain reaction, LCR) and amplification methods based on the use of Q-beta replicase. Also useful are strand displacement amplification (SDA), thermophilic SDA, and nucleic acid sequence based amplification (3SR or NASBA). These methods are well known and widely practiced in the art. See, e.g., U.S. Patents 4,683,195 and 4,683,202 and Innis et al., 1990 (for PCR); Wu and Wallace, 1989 (for LCR); U.S. Patents 5,270,184 and 5,455,166 and Walker et al., 1992 (for SDA); Spargo et al., 1996 (for thermophilic SDA) and U.S. Patent 5,409,818, Fahy et al., 1991 and Compton, 1991 for 3SR and NASBA. Reagents and hardware for conducting PCR are commercially available. Primers useful to amplify sequences from the *KCNQ2* or *KCNQ3* region are preferably complementary to, and hybridize specifically to sequences in the *KCNQ2* or *KCNQ3* region or in regions that flank a target region therein. *KCNQ2* or *KCNQ3* sequences generated by amplification may be sequenced directly. Alternatively, but less desirably, the amplified sequence(s) may be cloned prior to sequence analysis. A method for the direct cloning and sequence analysis of enzymatically amplified genomic segments has been described by Scharf et al., 1986.

"**Analyte polynucleotide**" and "**analyte strand**" refer to a single- or double-stranded polynucleotide which is suspected of containing a target sequence, and which may be present in a variety of types of samples, including biological samples.

"**Antibodies.**" The present invention also provides polyclonal and/or monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof, which are capable of specifically binding to the *KCNQ2* or *KCNQ3* polypeptide and fragments thereof or to polynucleotide sequences from the *KCNQ2* *KCNQ3* region. The term "**antibody**" is used both to refer to a homogeneous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Polypeptides may be prepared synthetically in a peptide synthesizer and coupled to a carrier molecule (e.g., keyhole limpet hemocyanin) and injected over several months into rabbits. Rabbit sera is tested for immunoreactivity to the *KCNQ2* or *KCNQ3* polypeptide or fragment. Monoclonal antibodies may be made by injecting mice with the protein polypeptides, fusion proteins or fragments thereof. Monoclonal antibodies will be screened by ELISA and tested for specific immunoreactivity with *KCNQ2* or *KCNQ3* polypeptide or fragments

thereof. See, Harlow and Lane, 1988. These antibodies will be useful in assays as well as pharmaceuticals.

Once a sufficient quantity of desired polypeptide has been obtained, it may be used for various purposes. A typical use is the production of antibodies specific for binding. These 5 antibodies may be either polyclonal or monoclonal, and may be produced by *in vitro* or *in vivo* techniques well known in the art. For production of polyclonal antibodies, an appropriate target immune system, typically mouse or rabbit, is selected. Substantially purified antigen is presented to the immune system in a fashion determined by methods appropriate for the animal and by other parameters well known to immunologists. Typical sites for injection are in footpads, 10 intramuscularly, intraperitoneally, or intradermally. Of course, other species may be substituted for mouse or rabbit. Polyclonal antibodies are then purified using techniques known in the art, adjusted for the desired specificity.

An immunological response is usually assayed with an immunoassay. Normally, such immunoassays involve some purification of a source of antigen, for example, that produced by the 15 same cells and in the same fashion as the antigen. A variety of immunoassay methods are well known in the art. See, e.g., Harlow and Lane, 1988, or Goding, 1986.

Monoclonal antibodies with affinities of  $10^{-8} \text{ M}^{-1}$  or preferably  $10^{-9}$  to  $10^{-10} \text{ M}^{-1}$  or stronger will typically be made by standard procedures as described, e.g., in Harlow and Lane, 1988 or Goding, 1986. Briefly, appropriate animals will be selected and the desired immunization protocol 20 followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the desired region of the antigen.

Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic 25 polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors. See Huse et al., 1989. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific 30 and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include U.S. Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345:



4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced (see U.S. Patent 4,816,567).

**"Binding partner"** refers to a molecule capable of binding a ligand molecule with high specificity, as for example, an antigen and an antigen-specific antibody or an enzyme and its inhibitor. In general, the specific binding partners must bind with sufficient affinity to immobilize the analyte copy/complementary strand duplex (in the case of polynucleotide hybridization) under the isolation conditions. Specific binding partners are known in the art and include, for example, biotin and avidin or streptavidin, IgG and protein A, the numerous, known receptor-ligand couples, and complementary polynucleotide strands. In the case of complementary polynucleotide binding partners, the partners are normally at least about 15 bases in length, and may be at least 40 bases in length. It is well recognized by those of skill in the art that lengths shorter than 15 (e.g., 8 bases), between 15 and 40, and greater than 40 bases may also be used. The polynucleotides may be composed of DNA, RNA, or synthetic nucleotide analogs. Further binding partners can be identified using, e.g., the two-hybrid yeast screening assay as described herein.

**A "biological sample"** refers to a sample of tissue or fluid suspected of containing an analyte polynucleotide or polypeptide from an individual including, but not limited to, e.g., plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, blood cells, tumors, organs, tissue and samples of *in vitro* cell culture constituents.

**"Encode"**. A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof. The anti-sense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

**"Isolated" or "substantially pure"**. An "isolated" or "substantially pure" nucleic acid (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native human sequence or protein, e.g., ribosomes, polymerases, many other human genome sequences and proteins. The term embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

"KCNQ2 Allele" refers to normal alleles of the KCNQ2 locus as well as alleles of KCNQ2 carrying variations that cause BFNC and/or rolandic epilepsy.

"KCNQ3 Allele" refers to normal alleles of the KCNQ3 locus as well as alleles of KCNQ3 carrying variations that cause BFNC and/or JME.

5 "KCNQ2 Locus", "KCNQ2 Gene", "KCNQ2 Nucleic Acids" or "KCNQ2 Polynucleotide" each refer to polynucleotides, all of which are in the *KCNQ2* region, that are likely to be expressed in normal tissue, certain alleles of which result in BFNC and/or rolandic epilepsy. The KCNQ2 locus is intended to include coding sequences, intervening sequences and regulatory elements controlling transcription and/or translation. The KCNQ2 locus is intended to include all  
10 allelic variations of the DNA sequence.

"KCNQ3 Locus", "KCNQ3 Gene", "KCNQ3 Nucleic Acids" or "KCNQ3 Polynucleotide" each refer to polynucleotides, all of which are in the *KCNQ3* region, that are likely to be expressed in normal tissue, certain alleles of which result in BFNC and/or JME. The KCNQ3 locus is intended to include coding sequences, intervening sequences and regulatory elements  
15 controlling transcription and/or translation. The KCNQ3 locus is intended to include all allelic variations of the DNA sequence.

These terms, when applied to a nucleic acid, refer to a nucleic acid which encodes a human KCNQ2 or KCNQ3 polypeptide, fragment, homolog or variant, including, e.g., protein fusions or deletions. The nucleic acids of the present invention will possess a sequence which is either derived  
20 from, or substantially similar to a natural KCNQ2- or KCNQ3-encoding gene or one having substantial homology with a natural KCNQ2- or KCNQ3-encoding gene or a portion thereof.

The *KCNQ2* or *KCNQ3* gene or nucleic acid includes normal alleles of the *KCNQ2* or *KCNQ3* gene, respectively, including silent alleles having no effect on the amino acid sequence of the KCNQ2 or KCNQ3 polypeptide as well as alleles leading to amino acid sequence variants of  
25 the KCNQ2 or KCNQ3 polypeptide that do not substantially affect its function. These terms also include alleles having one or more mutations which adversely affect the function of the KCNQ2 or KCNQ3 polypeptide. A mutation may be a change in the *KCNQ2* or *KCNQ3* nucleic acid sequence which produces a deleterious change in the amino acid sequence of the KCNQ2 or KCNQ3 polypeptide, resulting in partial or complete loss of KCNQ2 or KCNQ3 function, respectively, or  
30 may be a change in the nucleic acid sequence which results in the loss of effective KCNQ2 or KCNQ3 expression or the production of aberrant forms of the KCNQ2 or KCNQ3 polypeptide.

The *KCNQ2* or *KCNQ3* nucleic acid may be that shown in SEQ ID NO:1 (*KCNQ2*) or SEQ ID NO:6 (*KCNQ3*) or it may be an allele as described above or a variant or derivative differing from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to the nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

Thus, nucleic acid according to the present invention may include a sequence different from the sequence shown in SEQ ID NOs:1 and 6 yet encode a polypeptide with the same amino acid sequence as shown in SEQ ID NOs:2 (*KCNQ2*) and 7 (*KCNQ3*). That is, nucleic acids of the present invention include sequences which are degenerate as a result of the genetic code. On the other hand, the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in SEQ ID NOs:2 and 7. Nucleic acid encoding a polypeptide which is an amino acid sequence variant, derivative or allele of the amino acid sequence shown in SEQ ID NOs:2 and 7 is also provided by the present invention.

The *KCNQ2* or *KCNQ3* gene, respectively, also refers to (a) any DNA sequence that (i) hybridizes to the complement of the DNA sequences that encode the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:6 under highly stringent conditions (Ausubel et al., 1992) and (ii) encodes a gene product functionally equivalent to *KCNQ2* or *KCNQ3*, or (b) any DNA sequence that (i) hybridizes to the complement of the DNA sequences that encode the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:7 under less stringent conditions, such as moderately stringent conditions (Ausubel et al., 1992) and (ii) encodes a gene product functionally equivalent to *KCNQ2* or *KCNQ3*. The invention also includes nucleic acid molecules that are the complements of the sequences described herein.

The polynucleotide compositions of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a

designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The present invention provides recombinant nucleic acids comprising all or part of the  
5 *KCNQ2* or *KCNQ3* region. The recombinant construct may be capable of replicating autonomously in a host cell. Alternatively, the recombinant construct may become integrated into the chromosomal DNA of the host cell. Such a recombinant polynucleotide comprises a polynucleotide of genomic, cDNA, semi-synthetic, or synthetic origin which, by virtue of its origin or manipulation, 1) is not associated with all or a portion of a polynucleotide with which it is associated in nature; 2) is linked  
10 to a polynucleotide other than that to which it is linked in nature; or 3) does not occur in nature. Where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

Therefore, recombinant nucleic acids comprising sequences otherwise not naturally occurring are provided by this invention. Although the wild-type sequence may be employed, it will  
15 often be altered, e.g., by deletion, substitution or insertion. cDNA or genomic libraries of various types may be screened as natural sources of the nucleic acids of the present invention, or such nucleic acids may be provided by amplification of sequences resident in genomic DNA or other natural sources, e.g., by PCR. The choice of cDNA libraries normally corresponds to a tissue source which is abundant in mRNA for the desired proteins. Phage libraries are normally preferred, but  
20 other types of libraries may be used. Clones of a library are spread onto plates, transferred to a substrate for screening, denatured and probed for the presence of desired sequences.

The DNA sequences used in this invention will usually comprise at least about five codons (15 nucleotides), more usually at least about 7-15 codons, and most preferably, at least about 35 codons. One or more introns may also be present. This number of nucleotides is usually about the  
25 minimal length required for a successful probe that would hybridize specifically with a *KCNQ2*- or *KCNQ3*-encoding sequence. In this context, oligomers of as low as 8 nucleotides, more generally 8-17 nucleotides, can be used for probes, especially in connection with chip technology.

Techniques for nucleic acid manipulation are described generally, for example, in Sambrook et al., 1989 or Ausubel et al., 1992. Reagents useful in applying such techniques, such as restriction  
30 enzymes and the like, are widely known in the art and commercially available from such vendors as New England BioLabs, Boehringer Mannheim, Amersham, Promega, U. S. Biochemicals, New England Nuclear, and a number of other sources. The recombinant nucleic acid sequences used to

produce fusion proteins of the present invention may be derived from natural or synthetic sequences. Many natural gene sequences are obtainable from various cDNA or from genomic libraries using appropriate probes. See, GenBank, National Institutes of Health.

As used herein, a "**portion**" of the *KCNQ2* or *KCNQ3* locus or region or allele is defined  
5 as having a minimal size of at least about eight nucleotides, or preferably about 15 nucleotides, or more preferably at least about 25 nucleotides, and may have a minimal size of at least about 40 nucleotides. This definition includes all sizes in the range of 8-40 nucleotides as well as greater than 40 nucleotides. Thus, this definition includes nucleic acids of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400, 500 nucleotides, or nucleic acids having any number of nucleotides within these ranges  
10 of values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc., nucleotides), or nucleic acids having more than 500 nucleotides. The present invention includes all novel nucleic acids having at least 8 nucleotides derived from SEQ ID NO:1 or SEQ ID NO:6, its complement or functionally equivalent nucleic acid sequences. The present invention does not include nucleic acids which exist in the prior art. That is, the present invention includes all nucleic acids having at least 8 nucleotides derived  
15 from SEQ ID NO:1 or SEQ ID NO:6 with the proviso that it does not include nucleic acids existing in the prior art.

"**KCNQ2 protein**" or "**KCNQ2 polypeptide**" refers to a protein or polypeptide encoded by the *KCNQ2* locus, variants or fragments thereof. The term "polypeptide" refers to a polymer of amino acids and its equivalent and does not refer to a specific length of the product; thus, peptides,  
20 oligopeptides and proteins are included within the definition of a polypeptide. This term also does not refer to, or exclude modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages as well as other modifications known in the art, both  
25 naturally and non-naturally occurring. Ordinarily, such polypeptides will be at least about 50% homologous to the native *KCNQ2* sequence, preferably in excess of about 90%, and more preferably at least about 95% homologous. Also included are proteins encoded by DNA which hybridize under high or low stringency conditions, to *KCNQ2*-encoding nucleic acids and closely related polypeptides or proteins retrieved by antisera to the *KCNQ2* protein(s).

30 "**KCNQ3 protein**" or "**KCNQ3 polypeptide**" refers to a protein or polypeptide encoded by the *KCNQ3* locus, variants or fragments thereof. The term "polypeptide" refers to a polymer of amino acids and its equivalent and does not refer to a specific length of the product; thus, peptides,

oligopeptides and proteins are included within the definition of a polypeptide. This term also does not refer to, or exclude modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.). polypeptides with substituted linkages as well as other modifications known in the art, both naturally and non-naturally occurring. Ordinarily, such polypeptides will be at least about 50% homologous to the native KCNQ3 sequence, preferably in excess of about 90%, and more preferably at least about 95% homologous. Also included are proteins encoded by DNA which hybridize under high or low stringency conditions, to KCNQ3-encoding nucleic acids and closely related polypeptides or proteins retrieved by antisera to the KCNQ3 protein(s).

The KCNQ2 or KCNQ3 polypeptide may be that shown in SEQ ID NO:2 or SEQ ID NO:7 which may be in isolated and/or purified form, free or substantially free of material with which it is naturally associated. The polypeptide may, if produced by expression in a prokaryotic cell or produced synthetically, lack native post-translational processing, such as glycosylation. Alternatively, the present invention is also directed to polypeptides which are sequence variants, alleles or derivatives of the KCNQ2 or KCNQ3 polypeptide. Such polypeptides may have an amino acid sequence which differs from that set forth in SEQ ID NO:2 or SEQ ID NO:7 by one or more of addition, substitution, deletion or insertion of one or more amino acids. Preferred such polypeptides have KCNQ2 or KCNQ3 function.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, such as stability against proteolytic cleavage, without the loss of other functions or properties. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. Preferred substitutions are ones which are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and tyrosine, phenylalanine.

Certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules or binding sites on proteins interacting

with the KCNQ2 or KCNQ3 polypeptide. Since it is the interactive capacity and nature of a protein which defines that protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydrophobic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle, 1982). Alternatively, the substitution of like amino acids can be made effectively on the basis of hydrophilicity. The importance of hydrophilicity in conferring interactive biological function of a protein is generally understood in the art (U.S. Patent 4,554,101). The use of the hydrophobic index or hydrophilicity in designing polypeptides is further discussed in U.S. Patent 5,691,198.

The length of polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues.

**"Operably linked"** refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression.

The term **peptide mimetic** or **mimetic** is intended to refer to a substance which has the essential biological activity of the KCNQ2 or KCNQ3 polypeptide. A peptide mimetic may be a peptide-containing molecule that mimics elements of protein secondary structure (Johnson et al., 1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen, enzyme and substrate or scaffolding proteins. A peptide mimetic is designed to permit molecular interactions similar to the natural molecule. A mimetic may not be a peptide at all, but it will retain the essential biological activity of natural KCNQ2 or KCNQ3 polypeptide.

**"Probes"**. Polynucleotide polymorphisms associated with *KCNQ2* or *KCNQ3* alleles which predispose to BFNC, rolandic epilepsy or JME are detected by hybridization with a polynucleotide probe which forms a stable hybrid with that of the target sequence, under highly stringent to moderately stringent hybridization and wash conditions. If it is expected that the probes will be perfectly complementary to the target sequence, high stringency conditions will be used. Hybridization stringency may be lessened if some mismatching is expected, for example, if variants are expected with the result that the probe will not be completely complementary. Conditions are

chosen which rule out nonspecific/adventitious bindings, that is, which minimize noise. (It should be noted that throughout this disclosure, if it is simply stated that "stringent" conditions are used that is meant to be read as "high stringency" conditions are used.) Since such indications identify neutral DNA polymorphisms as well as mutations, these indications need further analysis to demonstrate  
5 detection of a *KCNQ2* or *KCNQ3* susceptibility allele.

Probes for *KCNQ2* alleles may be derived from the sequences of the *KCNQ2* region, its cDNA, functionally equivalent sequences, or the complements thereof. Probes for *KCNQ3* alleles may be derived from the sequences of the *KCNQ3* region, its cDNA, functionally equivalent sequences, or the complements thereof. The probes may be of any suitable length, which span all  
10 or a portion of the *KCNQ2* or *KCNQ3* region, and which allow specific hybridization to the region. If the target sequence contains a sequence identical to that of the probe, the probes may be short, e.g., in the range of about 8-30 base pairs, since the hybrid will be relatively stable under even highly stringent conditions. If some degree of mismatch is expected with the probe, i.e., if it is suspected that the probe will hybridize to a variant region, a longer probe may be employed which  
15 hybridizes to the target sequence with the requisite specificity.

The probes will include an isolated polynucleotide attached to a label or reporter molecule and may be used to isolate other polynucleotide sequences, having sequence similarity by standard methods. For techniques for preparing and labeling probes see, e.g., Sambrook et al., 1989 or Ausubel et al., 1992. Other similar polynucleotides may be selected by using homologous  
20 polynucleotides. Alternatively, polynucleotides encoding these or similar polypeptides may be synthesized or selected by use of the redundancy in the genetic code. Various codon substitutions may be introduced, e.g., by silent changes (thereby producing various restriction sites) or to optimize expression for a particular system. Mutations may be introduced to modify the properties of the polypeptide, perhaps to change the polypeptide degradation or turnover rate.

25 Probes comprising synthetic oligonucleotides or other polynucleotides of the present invention may be derived from naturally occurring or recombinant single- or double-stranded polynucleotides, or be chemically synthesized. Probes may also be labeled by nick translation, Klenow fill-in reaction, or other methods known in the art.

Portions of the polynucleotide sequence having at least about eight nucleotides, usually at  
30 least about 15 nucleotides, and fewer than about 9 kb, usually fewer than about 1.0 kb, from a polynucleotide sequence encoding *KCNQ2* or *KCNQ3* are preferred as probes. This definition therefore includes probes of sizes 8 nucleotides through 9000 nucleotides. Thus, this definition



includes probes of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400 or 500 nucleotides or probes having any number of nucleotides within these ranges of values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc., nucleotides), or probes having more than 500 nucleotides. The probes may also be used to determine whether mRNA encoding *KCNQ2* or *KCNQ3* is present in a cell or tissue. The present invention includes all novel probes having at least 8 nucleotides derived from SEQ ID NO:1 or SEQ ID NO:6, its complement or functionally equivalent nucleic acid sequences. The present invention does not include probes which exist in the prior art. That is, the present invention includes all probes having at least 8 nucleotides derived from SEQ ID NO:1 or SEQ ID NO:6 with the proviso that they do not include probes existing in the prior art.

Similar considerations and nucleotide lengths are also applicable to primers which may be used for the amplification of all or part of the *KCNQ2* or *KCNQ3* gene. Thus, a definition for primers includes primers of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400, 500 nucleotides, or primers having any number of nucleotides within these ranges of values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc. nucleotides), or primers having more than 500 nucleotides, or any number of nucleotides between 500 and 9000. The primers may also be used to determine whether mRNA encoding *KCNQ2* or *KCNQ3* is present in a cell or tissue. The present invention includes all novel primers having at least 8 nucleotides derived from the *KCNQ2* or *KCNQ3* locus for amplifying the *KCNQ2* or *KCNQ3* gene, its complement or functionally equivalent nucleic acid sequences. The present invention does not include primers which exist in the prior art. That is, the present invention includes all primers having at least 8 nucleotides with the proviso that it does not include primers existing in the prior art.

**"Protein modifications or fragments"** are provided by the present invention for *KCNQ2* or *KCNQ3* polypeptides or fragments thereof which are substantially homologous to primary structural sequence but which include, e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, e.g., with radionuclides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as <sup>32</sup>P, ligands which bind to labeled antiligands (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability

requirements, and available instrumentation. Methods of labeling polypeptides are well known in the art. See Sambrook et al., 1989 or Ausubel et al., 1992.

Besides substantially full-length polypeptides, the present invention provides for biologically active fragments of the polypeptides. Significant biological activities include ligand-binding, immunological activity and other biological activities characteristic of KCNQ2 or KCNQ3 polypeptides. Immunological activities include both immunogenic function in a target immune system, as well as sharing of immunological epitopes for binding, serving as either a competitor or substitute antigen for an epitope of the KCNQ2 or KCNQ3 protein. As used herein, "epitope" refers to an antigenic determinant of a polypeptide. An epitope could comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods of determining the spatial conformation of such amino acids are known in the art.

For immunological purposes, tandem-repeat polypeptide segments may be used as immunogens, thereby producing highly antigenic proteins. Alternatively, such polypeptides will serve as highly efficient competitors for specific binding. Production of antibodies specific for KCNQ2 or KCNQ3 polypeptides or fragments thereof is described below.

The present invention also provides for fusion polypeptides, comprising KCNQ2 or KCNQ3 polypeptides and fragments. Homologous polypeptides may be fusions between two or more KCNQ2 or KCNQ3 polypeptide sequences or between the sequences of KCNQ2 or KCNQ3 and a related protein. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. For example, ligand-binding or other domains may be "swapped" between different new fusion polypeptides or fragments. Such homologous or heterologous fusion polypeptides may display, for example, altered strength or specificity of binding. Fusion partners include immunoglobulins, bacterial  $\beta$ -galactosidase, trpE, protein A,  $\beta$ -lactamase, alpha amylase, alcohol dehydrogenase and yeast alpha mating factor. See Godowski et al., 1988.

Fusion proteins will typically be made by either recombinant nucleic acid methods, as described below, or may be chemically synthesized. Techniques for the synthesis of polypeptides are described, for example, in Merrifield, 1963.

**"Protein purification"** refers to various methods for the isolation of the KCNQ2 or KCNQ3 polypeptides from other biological material, such as from cells transformed with recombinant nucleic acids encoding KCNQ2 or KCNQ3, and are well known in the art. For example, such

polypeptides may be purified by immunoaffinity chromatography employing, e.g., the antibodies provided by the present invention. Various methods of protein purification are well known in the art, and include those described in Deutscher, 1990 and Scopes, 1982.

The terms "isolated", "substantially pure", and "substantially homogeneous" are used interchangeably to describe a protein or polypeptide which has been separated from components which accompany it in its natural state. A monomeric protein is substantially pure when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art which are utilized for purification.

A KCNQ2 or KCNQ3 protein is substantially free of naturally associated components when it is separated from the native contaminants which accompany it in its natural state. Thus, a polypeptide which is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

A polypeptide produced as an expression product of an isolated and manipulated genetic sequence is an "isolated polypeptide," as used herein, even if expressed in a homologous cell type. Synthetically made forms or molecules expressed by heterologous cells are inherently isolated molecules.

"**Recombinant nucleic acid**" is a nucleic acid which is not naturally occurring, or which is made by the artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions.

"Regulatory sequences" refers to those sequences normally within 100 kb of the coding region of a locus, but they may also be more distant from the coding region, which affect the expression of the gene (including transcription of the gene, and translation, splicing, stability or the like of the messenger RNA).

5        **"Substantial homology or similarity"**. A nucleic acid or fragment thereof is "substantially homologous" ("or substantially similar") to another if, when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least  
10 about 95-98% of the nucleotide bases.

To determine homology between two different nucleic acids, the percent homology is to be determined using the BLASTN program "BLAST 2 sequences". This program is available for public use from the National Center for Biotechnology Information (NCBI) over the Internet (<http://www.ncbi.nlm.nih.gov/gorf/bl2.html>) (Altschul et al., 1997). The parameters to be used are  
15 whatever combination of the following yields the highest calculated percent homology (as calculated below) with the default parameters shown in parentheses:

Program - blastn

Matrix - 0 BLOSUM62

Reward for a match - 0 or 1 (1)

20        Penalty for a mismatch - 0, -1, -2 or -3 (-2)

Open gap penalty - 0, 1, 2, 3, 4 or 5 (5)

Extension gap penalty - 0 or 1 (1)

Gap x\_dropoff - 0 or 50 (50)

Expect - 10

25        Along with a variety of other results, this program shows a percent identity across the complete strands or across regions of the two nucleic acids being matched. The program shows as part of the results an alignment and identity of the two strands being compared. If the strands are of equal length then the identity will be calculated across the complete length of the nucleic acids. If the strands are of unequal lengths, then the length of the shorter nucleic acid is to be used. If the  
30 nucleic acids are quite similar across a portion of their sequences but different across the rest of their sequences, the blastn program "BLAST 2 Sequences" will show an identity across only the similar portions, and these portions are reported individually. For purposes of determining homology

herein, the percent homology refers to the shorter of the two sequences being compared. If any one region is shown in different alignments with differing percent identities, the alignments which yield the greatest homology are to be used. The averaging is to be performed as in this example of SEQ ID NOs:20 and 21.

5 5'-ACCGTAGCTACGTACGTATATAGAAAGGGCGCGATCGTCGTCGCGTATGACGAC  
TTAGCATGC-3' (SEQ ID NO:20)

5'-ACCGGTAGCTACGTACGTTATTTAGAAAGGGGTGTGTGTGTGTGTGTAAACCGGG  
GTTTTTCGGGATCGTCCGTCGCGTATGACGACTTAGCCATGCACGGTATATCGTATTA  
GGACTAGCGATTGACTAG-3' (SEQ ID NO:21)

10 The program "BLAST 2 Sequences" shows differing alignments of these two nucleic acids depending upon the parameters which are selected. As examples, four sets of parameters were selected for comparing SEQ ID NOs:20 and 21 (gap x\_dropoff was 50 for all cases), with the results shown in Table 1. It is to be noted that none of the sets of parameters selected as shown in Table 1 is necessarily the best set of parameters for comparing these sequences. The percent homology  
15 is calculated by multiplying for each region showing identity the fraction of bases of the shorter strand within a region times the percent identity for that region and adding all of these together. For example, using the first set of parameters shown in Table 1, SEQ ID NO:20 is the short sequence (63 bases), and two regions of identity are shown, the first encompassing bases 4-29 (26 bases) of SEQ ID NO:20 with 92% identity to SEQ ID NO:21 and the second encompassing bases 39-59 (21  
20 bases) of SEQ ID NO:20 with 100% identity to SEQ ID NO:21. Bases 1-3, 30-38 and 60-63 (16 bases) are not shown as having any identity with SEQ ID NO:21. Percent homology is calculated as:  $(26/63)(92) + (21/63)(100) + (16/63)(0) = 71.3\%$  homology. The percents of homology calculated using each of the four sets of parameters shown are listed in Table 1. Several other combinations of parameters are possible, but they are not listed for the sake of brevity. It is seen  
25 that each set of parameters resulted in a different calculated percent homology. Because the result yielding the highest percent homology is to be used, based solely on these four sets of parameters one would state that SEQ ID NOs:20 and 21 have 87.1% homology. Again it is to be noted that use of other parameters may show an even higher homology for SEQ ID NOs:20 and 21, but for brevity not all the possible results are shown.

30 Alternatively, substantial homology or (similarity) exists when a nucleic acid or fragment thereof will hybridize to another nucleic acid (or a complementary strand thereof) under selective hybridization conditions, to a strand, or to its complement. Selectivity of hybridization exists when

TABLE 1

Parameter Values				Regions of identity (%)		Homology
Match	Mismatch	Open Gap	Extension Gap			
1	-2	5	1	4-29 of 20 and 5-31 of 21 (92%)	39-59 of 20 and 71-91 of 21 (100%)	71.3
1	-2	2	1	4-29 of 20 and 5-31 of 21 (92%)	33-63 of 20 and 64-96 of 21 (93%)	83.7
1	-1	5	1	-----	30-59 of 20 and 61-91 of 21 (93%)	44.3
1	-1	2	1	4-29 of 20 and 5-31 of 21 (92%)	30-63 of 20 and 61-96 of 21 (91%)	87.1

hybridization which is substantially more selective than total lack of specificity occurs. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, Kanehisa, 1984. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides.

Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. The stringency conditions are dependent on the length of the nucleic acid and the base composition of the nucleic acid and can be determined by techniques well known in the art. See, e.g., Wetmur and Davidson, 1968.

Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art.

The terms "**substantial homology**" or "**substantial identity**", when referring to polypeptides, indicate that the polypeptide or protein in question exhibits at least about 30% identity with an entire naturally-occurring protein or a portion thereof, usually at least about 70% identity, more usually at least about 80% identity, preferably at least about 90% identity, and more preferably at least about 95% identity.

Homology, for polypeptides, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wisconsin 53705. Protein analysis software matches similar sequences using measures of homology assigned to various substitutions, deletions and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine: valine, isoleucine, leucine: aspartic acid,

glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

"**Substantially similar function**" refers to the function of a modified nucleic acid or a modified protein, with reference to the wild-type *KCNQ2* or *KCNQ3* nucleic acid or wild-type  
5 *KCNQ2* or *KCNQ3* polypeptide. The modified polypeptide will be substantially homologous to the wild-type *KCNQ2* or *KCNQ3* polypeptide and will have substantially the same function. The modified polypeptide may have an altered amino acid sequence and/or may contain modified amino acids. In addition to the similarity of function, the modified polypeptide may have other useful properties, such as a longer half-life. The similarity of function (activity) of the modified  
10 polypeptide may be substantially the same as the activity of the wild-type *KCNQ2* or *KCNQ3* polypeptide. Alternatively, the similarity of function (activity) of the modified polypeptide may be higher than the activity of the wild-type *KCNQ2* or *KCNQ3* polypeptide. The modified polypeptide is synthesized using conventional techniques, or is encoded by a modified nucleic acid and produced using conventional techniques. The modified nucleic acid is prepared by conventional techniques.  
15 A nucleic acid with a function substantially similar to the wild-type *KCNQ2* or *KCNQ3* gene function produces the modified protein described above.

A polypeptide "**fragment**," "**portion**" or "**segment**" is a stretch of amino acid residues of at least about five to seven contiguous amino acids, often at least about seven to nine contiguous amino acids, typically at least about nine to 13 contiguous amino acids and, most preferably, at least  
20 about 20 to 30 or more contiguous amino acids.

The polypeptides of the present invention, if soluble, may be coupled to a solid-phase support, e.g., nitrocellulose, nylon, column packing materials (e.g., Sepharose beads), magnetic beads, glass wool, plastic, metal, polymer gels, cells, or other substrates. Such supports may take the form, for example, of beads, wells, dipsticks, or membranes.

25 "**Target region**" refers to a region of the nucleic acid which is amplified and/or detected. The term "**target sequence**" refers to a sequence with which a probe or primer will form a stable hybrid under desired conditions.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, and  
30 immunology. See, e.g., Maniatis et al., 1982; Sambrook et al., 1989; Ausubel et al., 1992; Glover, 1985; Anand, 1992; Guthrie and Fink, 1991. A general discussion of techniques and materials for



human gene mapping, including mapping of human chromosome 1, is provided, e.g., in White and Lalouel. 1988.

Preparation of recombinant or chemically synthesized

5 nucleic acids; vectors, transformation, host cells

Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natural or synthetic polynucleotide fragments coding for a desired fragment will be incorporated into recombinant polynucleotide constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the  
10 polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cell lines. The purification of nucleic acids produced by the methods of the present invention are described, e.g., in Sambrook et al., 1989 or Ausubel et al., 1992.

15 The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Carruthers (1981) or the triester method according to Matteucci and Caruthers (1981), and may be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and  
20 annealing the strands together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and  
25 translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Such vectors may be  
30 prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Sambrook et al., 1989 or Ausubel et al., 1992.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may include, when appropriate, those naturally associated with the *KCNQ2* or *KCNQ3* gene. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al., 1989 or Ausubel et al., 1992; see also, e.g., Metzger et al., 1988.

5 Many useful vectors are known in the art and may be obtained from such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. Promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, 10 enzymes responsible for maltose and galactose utilization, and others. Vectors and promoters suitable for use in yeast expression are further described in Hitzeman et al., EP 73,675A. Appropriate non-native mammalian promoters might include the early and late promoters from SV40 (Fiers et al., 1978) or promoters derived from murine Molony leukemia virus, mouse tumor virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. Insect promoters 15 may be derived from baculovirus. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also Enhancers and Eukaryotic Gene Expression, Cold Spring Harbor Press, Cold Spring Harbor, New York (1983). See also, e.g., U.S. Patent Nos. 5,691,198; 5,735,500; 5,747,469 and 5,436,146.

20 While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells which express the inserts. Typical selection 25 genes encode proteins that a) confer resistance to antibiotics or other toxic substances, e.g. ampicillin, neomycin, methotrexate, etc., b) complement auxotrophic deficiencies, or c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art.

30 The vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al., 1988), or the vectors can be introduced directly into host cells by methods well known in the

art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome); and other methods. See generally, Sambrook et al., 1989 and  
5 Ausubel et al., 1992. The introduction of the polynucleotides into the host cell by any method known in the art, including, *inter alia*, those described above, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

Large quantities of the nucleic acids and polypeptides of the present invention may be  
10 prepared by expressing the *KCNQ2* or *KCNQ3* nucleic acid or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used.

Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant,  
15 insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. Propagation of mammalian cells in culture is *per se* well known. See, Jakoby and Pastan (eds.) (1979). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38. BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be appropriate, e.g., to  
20 provide higher expression, desirable glycosylation patterns, or other features. An example of a commonly used insect cell line is SF9.

Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline  
25 or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

Prokaryotic or eukaryotic cells transformed with the polynucleotides of the present invention will be useful not only for the production of the nucleic acids and polypeptides of the present invention, but also, for example, in studying the characteristics of *KCNQ2* or *KCNQ3* polypeptide.  
30 The probes and primers based on the *KCNQ2* or *KCNQ3* gene sequence disclosed herein are used to identify homologous *KCNQ2* or *KCNQ3* gene sequences and proteins in other species.

These gene sequences and proteins are used in the diagnostic/prognostic, therapeutic and drug screening methods described herein for the species from which they have been isolated.

#### Methods of Use: Drug Screening

5 This invention is particularly useful for screening compounds by using the KCNQ2 or KCNQ3 polypeptide or binding fragment thereof in any of a variety of drug screening techniques.

The KCNQ2 or KCNQ3 polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, or borne on a cell surface. One method of drug screening utilizes eucaryotic or procaryotic host cells which are stably transformed with recombinant  
10 polynucleotides expressing the polypeptide or fragment, preferably in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, for the formation of complexes between a KCNQ2 or KCNQ3 polypeptide or fragment and the agent being tested, or examine the degree to which the formation of a complex between a KCNQ2 or KCNQ3 polypeptide or fragment and a known ligand is interfered with by the  
15 agent being tested.

Thus, the present invention provides methods of screening for drugs comprising contacting such an agent with a KCNQ2 or KCNQ3 polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the KCNQ2 or KCNQ3 polypeptide or fragment, or (ii) for the presence of a complex between the KCNQ2 or KCNQ3 polypeptide or fragment and a  
20 ligand, by methods well known in the art. In such competitive binding assays the KCNQ2 or KCNQ3 polypeptide or fragment is typically labeled. Free KCNQ2 or KCNQ3 polypeptide or fragment is separated from that present in a protein:protein complex, and the amount of free (i.e., uncomplexed) label is a measure of the binding of the agent being tested to KCNQ2 or KCNQ3 or its interference with KCNQ2(or KCNQ3):ligand binding, respectively. One may also measure the  
25 amount of bound, rather than free, KCNQ2 or KCNQ3. It is also possible to label the ligand rather than the KCNQ2 or KCNQ3 and to measure the amount of ligand binding to KCNQ2 or KCNQ3 in the presence and in the absence of the drug being tested.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the KCNQ2 or KCNQ3 polypeptides and is described in detail  
30 in Geysen (published PCT published application WO 84/03564). Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with KCNQ2 or KCNQ3 polypeptide

and washed. Bound KCNQ2 or KCNQ3 polypeptide is then detected by methods well known in the art.

Purified KCNQ2 or KCNQ3 can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to the polypeptide can be used  
5 to capture antibodies to immobilize the KCNQ2 or KCNQ3 polypeptide on the solid phase.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of specifically binding the KCNQ2 or KCNQ3 polypeptide compete with a test compound for binding to the KCNQ2 or KCNQ3 polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or  
10 more antigenic determinants of the KCNQ2 or KCNQ3 polypeptide.

The invention is particularly useful for screening compounds by using KCNQ2 or KCNQ3 protein in transformed cells, transfected oocytes or transgenic animals. The drug is added to the cells in culture or administered to a transgenic animal containing mutant KCNQ2 or KCNQ3 and the effect on the current of the potassium channel is compared to the current of a cell or animal  
15 containing the wild-type KCNQ2 or KCNQ3. Drug candidates which alter the current to a more normal level are useful for treating or preventing BFNC, rolandic epilepsy and JME.

The above screening methods are not limited to assays employing only KCNQ2 or KCNQ3 but are also applicable to studying KCNQ2- or KCNQ3-protein complexes. The effect of drugs on the activity of this complex is analyzed.

20 In accordance with these methods, the following assays are examples of assays which can be used for screening for drug candidates.

A mutant KCNQ2 or KCNQ3 (*per se* or as part of a fusion protein) is mixed with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type KCNQ2 or KCNQ3 binds. This mixing is performed in both the presence of a drug and the absence of the drug, and the amount  
25 of binding of the mutant KCNQ2 or KCNQ3 with the wild-type protein is measured. If the amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating BFNC, rolandic epilepsy or JME resulting from a mutation in *KCNQ2* or *KCNQ3*.

A wild-type KCNQ2 or KCNQ3 (*per se* or as part of a fusion protein) is mixed with a wild-  
30 type protein (*per se* or as part of a fusion protein) to which wild-type KCNQ2 or KCNQ3 binds. This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the wild-type KCNQ2 or KCNQ3 with the wild-type protein is measured. If the

amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating BFNC, rolandic epilepsy or JME resulting from a mutation in *KCNQ2* or *KCNQ3*.

A mutant protein, which as a wild-type protein binds to *KCNQ2* or *KCNQ3* (*per se* or as  
5 part of a fusion protein) is mixed with a wild-type *KCNQ2* or *KCNQ3* (*per se* or as part of a fusion protein). This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the mutant protein with the wild-type *KCNQ2* or *KCNQ3* is measured. If the amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating BFNC, rolandic epilepsy or JME resulting from a mutation  
10 in the gene encoding the protein.

The polypeptide of the invention may also be used for screening compounds developed as a result of combinatorial library technology. Combinatorial library technology provides an efficient way of testing a potential vast number of different substances for ability to modulate activity of a polypeptide. Such libraries and their use are known in the art. The use of peptide libraries is  
15 preferred. See, for example, WO 97/02048.

Briefly, a method of screening for a substance which modulates activity of a polypeptide may include contacting one or more test substances with the polypeptide in a suitable reaction medium, testing the activity of the treated polypeptide and comparing that activity with the activity of the polypeptide in comparable reaction medium untreated with the test substance or substances.  
20 A difference in activity between the treated and untreated polypeptides is indicative of a modulating effect of the relevant test substance or substances.

Prior to or as well as being screened for modulation of activity, test substances may be screened for ability to interact with the polypeptide, e.g., in a yeast two-hybrid system (e.g., Bartel et al., 1993; Fields and Song, 1989; Chevray and Nathans, 1992; Lee et al., 1995). This system may  
25 be used as a coarse screen prior to testing a substance for actual ability to modulate activity of the polypeptide. Alternatively, the screen could be used to screen test substances for binding to a *KCNQ2* or *KCNQ3* specific binding partner, or to find mimetics of the *KCNQ2* or *KCNQ3* polypeptide.

Following identification of a substance which modulates or affects polypeptide activity, the  
30 substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e., manufacture or formulation, or a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

Thus, the present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of polypeptide activity, in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a method comprising administration of such a composition comprising  
5 such a substance, a method comprising administration of such a composition to a patient, e.g., for treatment (which may include preventative treatment) of BFNC, rolandic epilepsy or JME, use of such a substance in the manufacture of a composition for administration, e.g., for treatment of BFNC, rolandic epilepsy or JME, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or  
10 carrier, and optionally other ingredients.

A substance identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use.

15 The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., pure peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic  
20 design, synthesis and testing is generally used to avoid randomly screening large numbers of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by  
25 systematically varying the amino acid residues in the peptide, e.g., by substituting each residue in turn. Alanine scans of peptide are commonly used to refine such peptide motifs. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g., stereochemistry, bonding, size and/or charge, using data from a range of sources,  
30 e.g., spectroscopic techniques, x-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted onto it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

#### Methods of Use: Nucleic Acid Diagnosis and Diagnostic Kits

In order to detect the presence of a *KCNQ2* or *KCNQ3* allele predisposing an individual to BFNC, rolandic epilepsy or JME, a biological sample such as blood is prepared and analyzed for the presence or absence of susceptibility alleles of *KCNQ2* or *KCNQ3*. In order to detect the presence of BFNC, rolandic epilepsy or JME, or as a prognostic indicator, a biological sample is prepared and analyzed for the presence or absence of mutant alleles of *KCNQ2* or *KCNQ3*. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic laboratories, or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

Initially, the screening method involves amplification of the relevant *KCNQ2* or *KCNQ3* sequences. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such screening methods include two-step label amplification methodologies that are well known in the art. Both PCR and non-PCR based screening strategies can detect target sequences with a high level of sensitivity.

The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is the polymerase chain reaction (PCR). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy



number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes.

When the probes are used to detect the presence of the target sequences the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence, e.g. denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region of human chromosome 20 for *KCNQ2* or to the targeted region of human chromosome 8 for *KCNQ3*. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. These factors are outlined in, for example, Maniatis et al., 1982 and Sambrook et al., 1989. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadruplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies, gold nanoparticles and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these

variations are reviewed in, e.g., Matthews and Kricka, 1988; Landegren et al., 1988; Mifflin, 1989; U.S. Patent 4,868,105; and in EPO Publication No. 225,807.

As noted above, non-PCR based screening assays are also contemplated in this invention. This procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output resulting in a  $10^3$ - $10^6$  increase in sensitivity. For an example relating to the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes, see Jablonski et al., 1986.

Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding *KCNQ2* or *KCNQ3*. Allele specific probes are also contemplated within the scope of this example and exemplary allele specific probes include probes encompassing the predisposing mutations of this disclosure.

In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. For methods for labeling nucleic acid probes according to this embodiment see Martin et al., 1990. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well known embodiment of this example is the biotin-avidin type of interactions. For methods for labeling nucleic acid probes and their use in biotin-avidin based assays see Rigby et al., 1977 and Nguyen et al., 1992.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes capable of detecting *KCNQ2* or *KCNQ3*. Thus, in one example to detect the presence of *KCNQ2* or *KCNQ3* in a cell sample, more than one probe complementary to the gene is employed and in particular the number of different probes is alternatively two, three, or five different nucleic acid probe sequences. In another example, to detect the presence of mutations in the *KCNQ2* or *KCNQ3* gene sequence in a patient,

more than one probe complementary to these genes is employed where the cocktail includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in *KCNQ2* or *KCNQ3*. In this embodiment, any number of probes can be used, and will preferably include probes corresponding to the major gene mutations identified as predisposing an individual to BFNC, rolandic epilepsy or JME.

#### Methods of Use: Peptide Diagnosis and Diagnostic Kits

The presence of BFNC, rolandic epilepsy or JME can also be detected on the basis of the alteration of wild-type *KCNQ2* or *KCNQ3* polypeptide. Such alterations can be determined by sequence analysis in accordance with conventional techniques. More preferably, antibodies (polyclonal or monoclonal) are used to detect differences in, or the absence of *KCNQ2* or *KCNQ3* peptides. Techniques for raising and purifying antibodies are well known in the art and any such techniques may be chosen to achieve the preparations claimed in this invention. In a preferred embodiment of the invention, antibodies will immunoprecipitate *KCNQ2* or *KCNQ3* proteins from solution as well as react with these proteins on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, antibodies will detect *KCNQ2* or *KCNQ3* proteins in paraffin or frozen tissue sections, using immunocytochemical techniques.

Preferred embodiments relating to methods for detecting *KCNQ2* or *KCNQ3* or their mutations include enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies. Exemplary sandwich assays are described by David et al., in U.S. Patent Nos. 4,376,110 and 4,486,530, hereby incorporated by reference.

#### Methods of Use: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide *in vivo*. See, e.g., Hodgson, 1991. In one approach, one first determines the three-dimensional structure of a protein of interest (e.g., *KCNQ2* or *KCNQ3* polypeptide) by x-ray crystallography, by computer modeling or most typically, by a combination of approaches. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous

proteins. An example of rational drug design is the development of HIV protease inhibitors (Erickson et al., 1990). In addition, peptides (e.g., KCNQ2 or KCNQ3 polypeptide) are analyzed by an alanine scan (Wells, 1991). In this technique, an amino acid residue is replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is  
5 analyzed in this manner to determine the important regions of the peptide.

It is also possible to isolate a target-specific antibody, selected by a functional assay, and then to solve its crystal structure. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody.  
10 As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacore.

Thus, one may design drugs which have, e.g., improved KCNQ2 or KCNQ3 polypeptide  
15 activity or stability or which act as inhibitors, agonists, antagonists, etc. of KCNQ2 or KCNQ3 polypeptide activity. By virtue of the availability of cloned *KCNQ2* and *KCNQ3* sequences, sufficient amounts of the KCNQ2 and KCNQ3 polypeptides may be made available to perform such analytical studies as x-ray crystallography. In addition, the knowledge of the KCNQ2 and KCNQ3 protein sequences provided herein will guide those employing computer modeling techniques in  
20 place of, or in addition to x-ray crystallography.

#### Methods of Use: Gene Therapy

According to the present invention, a method is also provided of supplying wild-type KCNQ2 or KCNQ3 function to a cell which carries a mutant *KCNQ2* or *KCNQ3* allele, respectively.  
25 Supplying such a function should allow normal functioning of the recipient cells. The wild-type gene or a part of the gene may be introduced into the cell in a vector such that the gene remains extrachromosomal. In such a situation, the gene will be expressed by the cell from the extrachromosomal location. More preferred is the situation where the wild-type gene or a part thereof is introduced into the mutant cell in such a way that it recombines with the endogenous  
30 mutant gene present in the cell. Such recombination requires a double recombination event which results in the correction of the gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector

may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation and viral transduction are known in the art, and the choice of method is within the competence of the practitioner.

As generally discussed above, the *KCNQ2* or *KCNQ3* gene or fragment, where applicable, 5 may be employed in gene therapy methods in order to increase the amount of the expression products of such gene in cells. It may also be useful to increase the level of expression of the *KCNQ2* or *KCNQ3* gene even in those persons in which the mutant gene is expressed at a "normal" level, but the gene product is not fully functional.

Gene therapy would be carried out according to generally accepted methods, for example, 10 as described by Friedman (1991) or Culver (1996). Cells from a patient would be first analyzed by the diagnostic methods described above, to ascertain the production of *KCNQ2* and/or *KCNQ3* polypeptide in the cells. A virus or plasmid vector (see further details below), containing a copy of the *KCNQ2* or *KCNQ3* gene linked to expression control elements and capable of replicating inside the cells, is prepared. The vector may be capable of replicating inside the cells. Alternatively, the 15 vector may be replication deficient and is replicated in helper cells for use in gene therapy. Suitable vectors are known, such as disclosed in U.S. Patent 5,252,479 and PCT published application WO 93/07282 and U.S. Patent Nos. 5,691,198; 5,747,469; 5,436,146 and 5,753,500. The vector is then injected into the patient. If the transfected gene is not permanently incorporated into the genome of each of the targeted cells, the treatment may have to be repeated periodically.

20 Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and nonviral transfer methods. A number of viruses have been used as gene transfer vectors or as the basis for repairing gene transfer vectors, including papovaviruses (e.g., SV40, Madzak et al., 1992), adenovirus (Berkner, 1992; Berkner et al., 1988; Gorziglia and Kapikian, 1992; Quantin et al., 1992; Rosenfeld et al., 1992; Wilkinson and 25 Akrigg, 1992; Stratford-Perricaudet et al., 1990; Schneider et al., 1998), vaccinia virus (Moss, 1992; Moss, 1996), adeno-associated virus (Muzyczka, 1992; Ohi et al., 1990; Russell and Hirata, 1998), herpesviruses including HSV and EBV (Margolskee, 1992; Johnson et al., 1992; Fink et al., 1992; Breakefield and Geller, 1987; Freese et al., 1990; Fink et al., 1996), lentiviruses (Naldini et al., 1996), Sindbis and Semliki Forest virus (Berglund et al., 1993), and retroviruses of avian 30 (Bandyopadhyay and Temin, 1984; Petropoulos et al., 1992), murine (Miller, 1992; Miller et al., 1985; Sorge et al., 1984; Mann and Baltimore, 1985; Miller et al., 1988), and human origin (Shimada et al., 1991; Helseth et al., 1990; Page et al., 1990; Buchschacher and Panganiban, 1992).

Most human gene therapy protocols have been based on disabled murine retroviruses, although adenovirus and adeno-associated virus are also being used.

Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham and van der Eb, 1973; Pellicer et al., 1980); mechanical techniques, for example microinjection (Anderson et al., 1980; Gordon et al., 1980; Brinster et al., 1981; Costantini and Lacy, 1981); membrane fusion-mediated transfer via liposomes (Felgner et al., 1987; Wang and Huang, 1989; Kaneda et al., 1989; Stewart et al., 1992; Nabel et al., 1990; Lim et al., 1991); and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990; Wu et al., 1991; Zenke et al., 1990; Wu et al., 1989; Wolff et al., 1991; Wagner et al., 1990; Wagner et al., 1991; Cotten et al., 1990; Curiel et al., 1992; Curiel et al., 1991). Viral-mediated gene transfer can be combined with direct *in vitro* gene transfer using liposome delivery, allowing one to direct the viral vectors to the tumor cells and not into the surrounding nondividing cells. Alternatively, the retroviral vector producer cell line can be injected into tumors (Culver et al., 1992). Injection of producer cells would then provide a continuous source of vector particles. This technique has been approved for use in humans with inoperable brain tumors.

In an approach which combines biological and physical gene transfer methods, plasmid DNA of any size is combined with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged. For other techniques for the delivery of adenovirus based vectors see Schneider et al. (1998) and U.S. Patent Nos. 5,691,198; 5,747,469; 5,436,146 and 5,753,500.

Liposome/DNA complexes have been shown to be capable of mediating direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer process is nonspecific, localized *in vivo* uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992).

Expression vectors in the context of gene therapy are meant to include those constructs containing sequences sufficient to express a polynucleotide that has been cloned therein. In viral expression vectors, the construct contains viral sequences sufficient to support packaging of the construct. If the polynucleotide encodes *KCNQ2* or *KCNQ3*, expression will produce *KCNQ2* or *KCNQ3*. If the polynucleotide encodes an antisense polynucleotide or a ribozyme, expression will produce the antisense polynucleotide or ribozyme. Thus in this context, expression does not require

that a protein product be synthesized. In addition to the polynucleotide cloned into the expression vector, the vector also contains a promoter functional in eukaryotic cells. The cloned polynucleotide sequence is under control of this promoter. Suitable eukaryotic promoters include those described above. The expression vector may also include sequences, such as selectable markers and other  
5 sequences described herein.

Gene transfer techniques which target DNA directly to brain tissue is preferred. Receptor-mediated gene transfer, for example, is accomplished by the conjugation of DNA (usually in the form of covalently closed supercoiled plasmid) to a protein ligand via polylysine. Ligands are chosen on the basis of the presence of the corresponding ligand receptors on the cell surface of the  
10 target cell/tissue type. These ligand-DNA conjugates can be injected directly into the blood if desired and are directed to the target tissue where receptor binding and internalization of the DNA-protein complex occurs. To overcome the problem of intracellular destruction of DNA, coinfection with adenovirus can be included to disrupt endosome function.

The therapy is as follows: patients who carry a *KCNQ2* or *KCNQ3* susceptibility allele are  
15 treated with a gene delivery vehicle such that some or all of their brain precursor cells receive at least one additional copy of a functional normal *KCNQ2* or *KCNQ3* allele, respectively. In this step, the treated individuals have reduced risk of BFNC, rolandic epilepsy and/or JME to the extent that the effect of the susceptible allele has been countered by the presence of the normal allele.

## 20 Methods of Use: Peptide Therapy

Peptides which have *KCNQ2* or *KCNQ3* activity can be supplied to cells which carry mutant or missing *KCNQ2* or *KCNQ3* alleles, respectively. Protein can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors. Alternatively, *KCNQ2* or *KCNQ3* polypeptide can be extracted from *KCNQ2*- or *KCNQ3*-producing mammalian cells.  
25 In addition, the techniques of synthetic chemistry can be employed to synthesize *KCNQ2* or *KCNQ3* protein. Any of such techniques can provide the preparation of the present invention which comprises the *KCNQ2* or *KCNQ3* protein. The preparation is substantially free of other human proteins. This is most readily accomplished by synthesis in a microorganism or *in vitro*.

Active *KCNQ2* or *KCNQ3* molecules can be introduced into cells by microinjection or by  
30 use of liposomes, for example. Alternatively, some active molecules may be taken up by cells, actively or by diffusion. Supply of molecules with *KCNQ2* or *KCNQ3* activity should lead to partial reversal of BFNC, rolandic epilepsy and/or JME. Other molecules with *KCNQ2* or *KCNQ3*

activity (for example, peptides, drugs or organic compounds) may also be used to effect such a reversal. Modified polypeptides having substantially similar function are also used for peptide therapy.

#### 5 Methods of Use: Transformed Hosts

Animals for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant *KCNQ2* and/or *KCNQ3* alleles, usually from a second animal species, as well as insertion of disrupted homologous genes. Alternatively, the endogenous *KCNQ2* or *KCNQ3* gene of the animals may be  
10 disrupted by insertion or deletion mutation or other genetic alterations using conventional techniques (Capecchi, 1989; Valancius and Smithies, 1991; Hasty et al., 1991; Shinkai et al., 1992; Mombaerts et al., 1992; Philpott et al., 1992; Snouwaert et al., 1992; Donehower et al., 1992). After test substances have been administered to the animals, the presence of BFNC, rolandic epilepsy or JME must be assessed. If the test substance prevents or suppresses the appearance of BFNC,  
15 rolandic epilepsy or JME, then the test substance is a candidate therapeutic agent for treatment of BFNC, rolandic epilepsy or JME. These animal models provide an extremely important testing vehicle for potential therapeutic products.

The identification of the association between the *KCNQ2* and *KCNQ3* gene mutations and BFNC, rolandic epilepsy and JME permits the early presymptomatic screening of individuals to  
20 identify those at risk for developing BFNC, rolandic epilepsy or JME. To identify such individuals, *KCNQ2* and/or *KCNQ3* alleles are screened for mutations either directly or after cloning the alleles. The alleles are tested for the presence of nucleic acid sequence differences from the normal allele using any suitable technique, including but not limited to, one of the following methods: fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single  
25 stranded conformation analysis (SSCP), linkage analysis, RNase protection assay, allele specific oligonucleotide (ASO), dot blot analysis and PCR-SSCP analysis. Also useful is the recently developed technique of DNA microchip technology. For example, either (1) the nucleotide sequence of both the cloned alleles and normal *KCNQ2* or *KCNQ3* gene or appropriate fragment (coding sequence or genomic sequence) are determined and then compared, or (2) the RNA  
30 transcripts of the *KCNQ2* or *KCNQ3* gene or gene fragment are hybridized to single stranded whole genomic DNA from an individual to be tested, and the resulting heteroduplex is treated with



Ribonuclease A (RNase A) and run on a denaturing gel to detect the location of any mismatches. Two of these methods can be carried out according to the following procedures.

The alleles of the *KCNQ2* or *KCNQ3* gene in an individual to be tested are cloned using conventional techniques. For example, a blood sample is obtained from the individual. The  
5 genomic DNA isolated from the cells in this sample is partially digested to an average fragment size of approximately 20 kb. Fragments in the range from 18-21 kb are isolated. The resulting fragments are ligated into an appropriate vector. The sequences of the clones are then determined and compared to the normal *KCNQ2* or *KCNQ3* gene.

Alternatively, polymerase chain reactions (PCRs) are performed with primer pairs for the  
10 5' region or the exons of the *KCNQ2* or *KCNQ3* gene. PCRs can also be performed with primer pairs based on any sequence of the normal *KCNQ2* or *KCNQ3* gene. For example, primer pairs for one of the introns can be prepared and utilized. Finally, RT-PCR can also be performed on the mRNA. The amplified products are then analyzed by single stranded conformation polymorphisms (SSCP) using conventional techniques to identify any differences and these are then sequenced and  
15 compared to the normal gene sequence.

Individuals can be quickly screened for common *KCNQ2* or *KCNQ3* gene variants by amplifying the individual's DNA using suitable primer pairs and analyzing the amplified product, e.g., by dot-blot hybridization using allele-specific oligonucleotide probes.

The second method employs RNase A to assist in the detection of differences between the  
20 normal *KCNQ2* or *KCNQ3* gene and defective genes. This comparison is performed in steps using small (~500 bp) restriction fragments of the *KCNQ2* or *KCNQ3* gene as the probe. First, the *KCNQ2* or *KCNQ3* gene is digested with a restriction enzyme(s) that cuts the gene sequence into fragments of approximately 500 bp. These fragments are separated on an electrophoresis gel, purified from the gel and cloned individually, in both orientations, into an SP6 vector (e.g., pSP64  
25 or pSP65). The SP6-based plasmids containing inserts of the *KCNQ2* or *KCNQ3* gene fragments are transcribed *in vitro* using the SP6 transcription system, well known in the art, in the presence of [ $\alpha$ -<sup>32</sup>P]GTP, generating radiolabeled RNA transcripts of both strands of the gene.

Individually, these RNA transcripts are used to form heteroduplexes with the allelic DNA using conventional techniques. Mismatches that occur in the RNA:DNA heteroduplex, owing to  
30 sequence differences between the *KCNQ2* or *KCNQ3* fragment and the *KCNQ2* or *KCNQ3* allele subclone from the individual, result in cleavage in the RNA strand when treated with RNase A. Such mismatches can be the result of point mutations or small deletions in the individual's allele.

Cleavage of the RNA strand yields two or more small RNA fragments, which run faster on the denaturing gel than the RNA probe itself.

Any differences which are found, will identify an individual as having a molecular variant of the *KCNQ2* or *KCNQ3* gene and the consequent presence of BFNC, rolandic epilepsy or JME.

5 These variants can take a number of forms. The most severe forms would be frame shift mutations or large deletions which would cause the gene to code for an abnormal protein or one which would significantly alter protein expression. Less severe disruptive mutations would include small in-frame deletions and nonconservative base pair substitutions which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic to an acidic  
10 amino acid or vice versa, from a hydrophobic to hydrophilic amino acid or vice versa, or other mutations which would affect secondary or tertiary protein structure. Silent mutations or those resulting in conservative amino acid substitutions would not generally be expected to disrupt protein function.

Genetic testing will enable practitioners to identify individuals at risk for BFNC, rolandic  
15 epilepsy or JME, at, or even before, birth. Presymptomatic diagnosis of these epilepsies will enable prevention of these disorders. Finally, this invention changes our understanding of the cause and treatment of BFNC, rolandic epilepsy and JME. It is possible, for example, that potassium channel opening agents will reduce the risk of seizures in patients with *KCNQ2* or *KCNQ3* mutations.

## 20 Pharmaceutical Compositions and Routes of Administration

The *KCNQ2* and *KCNQ3* polypeptides, antibodies, peptides and nucleic acids of the present invention can be formulated in pharmaceutical compositions, which are prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA). The composition may  
25 contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may comprise, in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for  
30 administration, e.g., intravenous, oral, intrathecal, epineural or parenteral.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing

the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, WO 96/11698.

For parenteral administration, the compound may be dissolved in a pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

The active agent is preferably administered in a therapeutically effective amount. The actual amount administered, and the rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc., is within the responsibility of general practitioners or specialists, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in Remington's Pharmaceutical Sciences.

Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

Instead of administering these agents directly, they could be produced in the target cell, e.g. in a viral vector such as described above or in a cell based delivery system such as described in U.S. Patent No. 5,550,050 and published PCT application Nos. WO 92/19195, WO 94/25503, WO

95/01203. WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635. designed for implantation in a patient. The vector could be targeted to the specific cells to be treated, or it could contain regulatory elements which are more tissue specific to the target cells. The cell based delivery system is designed to be implanted in a patient's body at the desired target site and contains a coding sequence for the active agent. Alternatively, the agent could be administered in a precursor form for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. See for example, EP 425,731A and WO 90/07936.

The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

#### EXAMPLE 1 Southern Blot Analysis

15

Five micrograms of genomic DNA were cut with *TaqI* and transferred to a nylon membrane. Filters were hybridized overnight at 65°C in PEG hyb (7% PEG, 10% SDA, 50 mM sodium phosphate and 200 µg/ml total human DNA) with the D20S24 plasmid probe labeled by random priming (Stratagene). Filters were washed at 2 x SSC, 0.1% SDS twice at room temperature followed by one wash in 0.5 x SSC, 0.1% SDS at 65°C.

#### EXAMPLE 2 Fluorescence in situ Hybridization

25

Chromosomes from transformed lymphocytes were prepared using a 30 minute ethidium bromide treatment followed by 3 hours in colcemid. Cells were then pelleted and resuspended in hypotonic solution (0.75 M KCl) for 20 minutes followed by the addition of four to five drops of fresh fixative (3:1 methanol:acetic acid). Cells were again pelleted, vortexed then carefully resuspended in fixative. After three washes in fixative, metaphases were stored at 4°C. Four hundred ng probe was labeled with biotin and hybridized to slides of metaphase spreads using standard hybridization procedures. Probes were then fluorescently tagged with avidin-FITC (Vector) and the signal intensified using biotin-labeled anti-avidin followed by avidin-FITC. The chromosomes were then counterstained using DAPI and visualized using a Zeiss Axioplan

30

Fluorescent microscope equipped with FITC, DAPI and triple band pass filter sets. Images were captured by computer using Applied Imaging (Pittsburgh, PA) software Probevision and photographs printed on a Kodak XL 7700 color image printer.

5

### EXAMPLE 3

#### Localization of KCNQ2

Linkage analysis in a large kindred demonstrated that a gene responsible for BFNC maps to chromosome 20q13.3 close to the markers D20S20 and D20S19 (Leppert et al., 1989). Following the initial report, two centers confirmed linkage of BFNC to the same two genetic markers on  
10 chromosome 20, termed the EBN1 (epilepsy benign neonatal type 1) locus (Ryan et al., 1991; Malafosse et al., 1992). A more distal marker, D20S24, shows complete co-segregation with the BFNC phenotype in chromosome 20 linked families. Finding a distal flanking marker for the BFNC locus has not been successful probably because of its proximity to the telomere. This telomeric region is characterized by a high recombination rate between markers when compared to the  
15 physical distance (Steinlein et al., 1992). In fact, Steinlein et al. have demonstrated that the three markers D20S19, D20S20 and D20S24 are contained on the same 450 Mb Mlu I restriction fragment (Steinlein et al., 1992).

A second chromosomal locus, EBN2, has also been identified for BFNC. Lewis et al. (1993) demonstrated linkage to markers on chromosome 8q24 in a single Hispanic family affected with  
20 BFNC. Evidence for this second locus was also reported in a Caucasian pedigree (Steinlein et al., 1995). All of the families in the present study show linkage to chromosome 20q markers with LOD scores of greater than 3.0 or have probands with clinical manifestations consistent with BFNC (Leppert et al., 1993). To find the gene responsible for BFNC, we narrowed the BFNC region with a sub-microscopic deletion in a single family, identified candidate cDNAs in this deletion, and then  
25 searched for mutations in other BFNC families.

Evidence for a small deletion came first from a genotypic observation with a three allele, RFLP marker, D20S24. Analysis of one family, kindred 1547, revealed that a null allele occurred exclusively in those individuals with BFNC and in two individuals previously shown to be non-penetrant with the VNTR markers D20S20 and D20S19 (Figure 1). The existence of a deletion co-  
30 segregating with the BFNC phenotype in this family was confirmed by fluorescence in situ hybridization (FISH) in cell lines of kindred 1547 individuals using as probes, the D20S24 plasmid and two genomic P1 clones containing this marker.

To confirm the presence of a deletion, two overlapping genomic P1 clones, P1-KO9-6b and P1-KO9-7, each of approximately 80 kb in size and each of which contains the D20S24 marker, were obtained and these were hybridized to cell lines of kindred 1547 BFNC affected individuals. When metaphase spread chromosomes are hybridized with P1-KO9-7 and P1-KO9-6b, both chromosome 20 homologs give signals on two sister chromatids. However when the 12 kb probe D20S24 is hybridized only signal from the one chromosome homolog is observed in 75% of metaphase spreads examined. The remaining minority of cells showed no hybridization for the 12 kb D20S24 probe (Figure 2). The plasmid containing the D20S24 marker was a kind gift from J. Weissenbach.

While the 12 kb D20S24 probe was deleted on one chromosome in affected individuals, the overlapping P1 clones of 80 kb in size, and which together span approximately 130 kb, showed a positive FISH signal indicating that the deletion is smaller than 130 kb (Figure 2).

#### EXAMPLE 4

##### Isolation and Characterization of *KCNQ2* Clones

Using the same probes as in Example 3, cDNAs in the region of the deletion were identified by screening a fetal brain cDNA library. Three of the cDNAs isolated showed significant homology to *KCNQ1*, the chromosome 11 potassium channel gene responsible for the Long QT syndrome and the Jervell and Lange-Nielsen cardicauditory syndrome (Wang et al., 1996; Altschul et al., 1990; Neyroud et al., 1997).

A fetal brain cDNA library (Stratagene) ( $10^6$  clones) was probed with inserts from P1-KO9-6b and P1-KO9-7 and the plasmid D20S24. Hybridizations were performed in 5 x SSC, 10 x Denhardt's, 0.1 M sodium phosphate (pH 6.7), 100 µg/mL salmon sperm DNA, 0.1% SDS and 50% formamide. Blots were washed in 2 x SSC, 0.1% SDS twice at room temperature followed by one wash in 0.5 x SSC, 0.1% SDS at 42°C.

A single cDNA isolated with D20S24, cIPK, showed 75% homology to amino acids 511-562 of *KCNQ1*; a second probing of the fetal brain cDNA library using the probe P1-KO9-6b resulted in the isolation of two additional cDNAs, c6b-6 and c6b-12, which showed significant homology with *KCNQ1* amino acids 398-406 and 354-378, respectively (Altschul et al., 1990; Wang et al., 1996; Neyroud et al., 1997).

Additional sequence encoding this BFNC gene, named *KVEBNI* (now *KCNQ2*) after the OMIM locus name, was obtained from RACE experiments using adaptor-ligated double-stranded

cDNA from fetal and adult brain tissue and from other cDNA clones isolated from a temporal cortex cDNA library.

To identify the full length gene, 5' and 3' RACE were performed on adaptor-ligated fetal and adult brain cDNA (Clontech) using primers within c6b-6 and cIPK and screening a temporal cortex  
5 cDNA library (Stratagene) with sequence flanking cIPK. Unprocessed cDNAs were repeatedly isolated from cDNA libraries and RACE experiments. The longest transcript isolated from brain was 1455 nucleotides long and was obtained using 5' RACE and extended from the S1 domain (amino acid 100) to the 3' conserved C-terminal domain (amino acid 585).

Composite clones encoding 872 amino acids of the KCNQ2 gene have been isolated (Figure  
10 3). The cDNA sequence for *KCNQ2* is shown as SEQ ID NO:1 and the amino acid sequence for KCNQ2 is shown as SEQ ID NO:2. The putative initiator methionine lies within a region similar to the Kozak consensus sequence (Kozak, 1987). KCNQ2 encodes a highly conserved six transmembrane motif as well as a pore region that are the hallmarks of a K<sup>+</sup> ion channel gene. The S2, S3 and S4 transmembrane regions also contain charged amino acids that are found in all  
15 members of the K<sup>+</sup> channel subfamilies, including *Shaker*, *Shab*, *Shaw* and *Shal*. A search of Genbank with KCNQ2 sequence shows identical nucleotide sequence to HNSPC (Accession # D82346), a 393 amino acid putative potassium channel cDNA isolated from a human neuroblastoma cell line (Yokoyama et al., 1996). However, the last 21 amino acids of HNSPC including a stop codon are encoded by a sequence that in *KCNQ2* is intronic. A search of the human expressed  
20 sequence tag database (dbest) shows seven different clones encoding portions of KCNQ2. Wei et al. have identified a gene from *C. elegans*, *nKQT1*, that appears to be a homolog of *KCNQ2* (Wei et al., 1996). This group also described the human EST homolog of *nKQT1*, *hKQT2*, which is a partial clone of *KCNQ2* (Wei et al., 1996). In addition to the six transmembrane domains and the pore, a small region 5' of transmembrane domain S1 is also conserved between KCNQ2, KCNQ3,  
25 KCNQ1 and nKQT1. Unlike other K<sup>+</sup> channel subfamilies, the C-terminal domain appears to contain highly conserved residues as shown in Figure 3 for KCNQ2, KCNQ3, nKQT1 and KCNQ1. The poly A tail for *KCNQ2* has not been identified to date.

### EXAMPLE 5

30

#### Northern Blot Analysis

The *KCNQ2* cDNA hybridizes to transcripts approximately 1.5, 3.8 and 9.5 kb in size on Northern blots made from brain. Multiple Tissue Northern (Clontech) of fetal and adult brain were

probed with a RACE product containing transmembrane domains S1 through S6 of KCNQ2. The 1.5 and 9.5 kb transcripts appear to be expressed in both adult and fetal brain. The 3.8 kb transcript is expressed in select areas from adult brain, particularly in the temporal lobe and the putamen.

5

### EXAMPLE 6

#### Mutational Analysis of KCNQ2

Mutational analysis of *KCNQ2* was performed on one affected individual from each of our 12 BFNC families. Coding regions from S1 to S6 and conserved regions in the 3' end of *KCNQ2* were amplified by PCR using primers within introns and analyzed by SSCP (Novex) using 20% TBE gels run at 4°C. The exon-intron boundaries were identified by sequencing products obtained by exon-exon PCR on genomic P1 clones or directly from RACE products which contained unprocessed transcripts. PCR products showing variants seen on SSCP were either cloned and sequenced or reamplified with M13 reverse and M13 universal-tailed primers and sequenced directly on an ABI 373 or 377 using dye-primer chemistry.

15 In addition to the substantial deletion in kindred 1547, mutations were identified in five other BFNC families. Mutational analysis was carried out by first screening probands for SSCP variants and then sequencing each individual's DNA to determine the basis for the molecular variation. Mutations identified include two missense mutations, two frameshift mutations and one splice site mutation (Table 2). Later analyses resulted in the finding of four more BFNC families with 20 mutations in *KCNQ2*. These include two nonsense mutations (families K1525 and K4443), an insertion resulting in a frameshift which results in readthrough beyond the normal stop codon (K3963), and a missense mutation (K4516). These latter 4 mutations are listed in Table 2.

The splice site variant occurs in an intron which occurs between two exons encoding amino acid residue 544. The first exon includes the TG at the start of codon 544 and the following exon 25 includes the final T of codon 544. The sequence at the 3' end of the intron (shown in lower case letters) and continuing into the exon region (shown in upper case letters) encoding the end of codon 544 and codons 545-546 is: 5'-tgcagTGTCATG-3' (SEQ ID NO:5). The "g" at position 5 of SEQ ID NO:5 is mutated to an "a" in kindred K3933.

None of the mutations seen in the first six families identified was seen in SSCP analysis of 30 our panel of 70 unrelated, unaffected individuals. Furthermore, mutations were shown to segregate completely with affection status in all of the BFNC families where mutations were identified. In the case of the splice site mutation in kindred 3933 only the proband was sampled. An example



Table 2Mutations in the *KCNQ2* Gene in BFNC Families

	Mutation at Amino Acid	Region	Kindred	Controls	Nucleotide Change
5	large deletion	not available	K1547	70	not available
	frameshift at 283	pore	K1504	70	insert GT between nucleotides 975 and 976 of SEQ ID NO:1
	Y284C	pore	K3904	70	A→G at base 978 of SEQ ID NO:1
	A306T	S6	K1705	70	G→A at base 1043 of SEQ ID NO:1
	Q323Stop	C-terminal	K4443	--	C→T at base 1094 of SEQ ID NO:1
10	R333Q	C-terminal	K4516	--	G→A at base 1125 of SEQ ID NO:1
	R448Stop	C-terminal	K1525	--	C→T at base 1469 of SEQ ID NO:1
	frameshift at 522	C-terminal	K3369	70	delete bases 1691 through 1703 of SEQ ID NO:1
	splice site variant	C-terminal	K3933	70	g→a at 3' end of intron which occurs between bases 1758 and 1759 of SEQ ID NO:1
	frameshift at 867	C-terminal	K3963	70	insert GGGCC after base 2736 of SEQ ID NO:1

15

of this segregation is shown in Figure 4 for the two base-pair insertion identified in kindred 1504; all 11 affected members of the kindred have the SSCP variant and all seven unaffected individuals have wild type SSCP bands.

Of the four families (K1525, K3963, K4443 and K4516) which have been more recently  
5 found to have *KCNQ2* mutations, three (K1525, K4443 and K4516) were found through direct sequencing and the mutation co-segregated in the family when other affected members were available for study. The mutation in K3963 was found via SSCP screening and this mutation was not detected in a panel of 70 normal, i.e., non BFNC, individuals. This mutation was found to co-segregate with affected individuals in family K3963. The wild-type gene includes two sets of  
10 GGGCC at bases 2727-2736 of SEQ ID NO:1. The sequence found in K3963 is three sets of GGGCC as a result of an insertion of GGGCC into this region. This results in the gene encoding the first 870 amino acids of the wild-type followed by an additional 60 amino acids of new sequence (amino acid residues 871 and 872 of the wild-type being replaced by the first 2 of the 60 additional amino acid residues). The gene including the 5 base insertion is shown as SEQ ID NO:95 and the  
15 protein encoded by this mutated gene is shown as SEQ ID NO:96.

Family K4443 has 6 BFNC affected individuals and two of these individuals have in addition seizures later in childhood that are classified as benign epilepsy with centrotemporal spikes (BERS), or rolandic epilepsy. The DNA of two affected individuals in this family was examined. The Q323Stop mutation is found in one of the affected individuals that expresses BFNC only and  
20 in one individual which has both BFNC and BERS or rolandic epilepsy, which developed later in childhood after the newborn seizures. This finding directly implicates the *KCNQ2* gene on chromosome 20 in causing rolandic epilepsy. Rolandic epilepsy, or BERS, is a common childhood epilepsy and may account for 25% of all school age epilepsy. This is a genetic disorder that inherits as an autosomal dominant with reduced penetrance. It is possible that several genes may cause the  
25 rolandic phenotype, but this finding strongly suggests that at least some of the rolandic epilepsies will be caused by defects in *KCNQ2*, a potentially important finding.

Two neutral polymorphisms were identified in the *KCNQ2* gene. One polymorphism is in codon 304 (TTC to TTT) in the S6 transmembrane domain and was seen in 10 of 71 controls who were each heterozygous (allelic frequency of 7.0%). The second polymorphism is in codon 573  
30 (GCC to GCT) in the 3' region and was observed in 1 of 87 controls individuals as a heterozygote (allelic frequency of 0.57%).

It is predicted that the splice site mutation in the conserved 3' region of *KCNQ2* and the two frameshift mutations, one in the pore region and one before the highly conserved 3' region, lead to altered protein products. In the case of the 283insGT pore mutation a predicted stop codon is found 36 amino acids downstream and in the case of the 522del13 3' mutation a predicted stop codon is found two amino acids downstream. Also, the two bp insertion mutation, 283insGT, would lead to a completely altered S6 transmembrane domain. While the breakpoints of the kindred 1547 deletion have not been determined, it is known that the 12 kb plasmid which includes the RFLP marker locus, D20S24, contains 80 codons (residues 509 to 588 of *KCNQ2*) of sequence from the highly conserved 3' region of the *KCNQ2* gene, indicating that at least this portion of the gene is deleted in kindred 1547 affected individuals. The two missense mutations in families K3904 and K1705 change amino acid residues in key functional domains, the pore and S6 domain.

Ten unique mutations have been identified in *KCNQ2* to date. The mutation defined by a 13 base pair deletion at amino acid 522 in kindred 3369 is of interest in that there is a greater variation in the reported clinical ages of onset within this family when compared to typical BFNC families. In kindred 3369, three individuals had onset of seizures within the first 2 weeks of life, while three individuals had initial onset of seizures at 3, 4, and 5 months of age.

The mutation in the BFNC kindred 1705 is an alanine to threonine substitution in the S6 transmembrane segment. This alanine residue is conserved in all members of the *Shaker*, *Shab*, *Shaw* and *Shal* subfamilies of potassium channels identified to date (Lopez et al., 1994; Nakamura et al., 1997; Tytgat, 1994). The *KCNQ1* gene, which the *KCNQ2* ion channel gene is most closely related to, also contains an alanine in this position. In six unrelated LQT1 families, the disease-causing mutation occurs at this same position where the alanine is changed to a valine (Wang et al., 1996; Russell et al., 1996). This S6 transmembrane domain has been shown to be involved in K<sup>+</sup> ion permeation in the *Shaker* subtype (Lopez et al., 1994) and may serve a similar function in *KCNQ2*. The C-terminal region appears to be important for gene function because a 13 bp deletion, a splice site mutation, a missense mutation, a nonsense mutation, and an insertion all produce an epileptic phenotype in separate BFNC families (see Table 2 and Figure 3). Interestingly, this same region is known to have a deletion-insertion mutation in *KCNQ1* in individuals with the Jervell and Lange-Nielsen recessive form of LQT and associated deafness (Neyroud et al., 1997). Disease-causing mutations in the C-terminal region further argue for a functional protein encoded by the *KCNQ2* gene rather than the shorter HNSPC clone.

The pore region of K<sup>+</sup> ion channels belonging to the same structural class have been characterized extensively by mutational analysis. The two base-pair insertion observed in kindred 1504 occurs immediately after the universally conserved GYG motif. An insertion here not only alters the length of the pore that is believed to be crucial for function (Nakamura et al., 1997; Tytgat, 5 1994) but also modifies the signature sequence of the pore and produces a truncated protein.

In infants of families that have been linked to the chromosome 20 form of BFNC, EEG recordings show initial suppression of activity throughout the brain followed by generalized discharges of spikes and slow waves (Ronen et al., 1993; Plouin, 1994; Hauser and Kurland, 1975). It is therefore not surprising to find that the *KCNQ2* gene is expressed in multiple brain areas in 10 adults. Cortical regions as well as sub-cortical areas, such as the thalamus and caudate nucleus, contain multiple size transcripts of *KCNQ2* (data not shown). It is possible that this expression pattern is also the same in the newborn infant.

The close homology (60% identity and 70% similarity of amino acids) of *KCNQ2* to *KCNQ1* and to the *C. elegans nKQT1* gene and the reduced homology of these channels to the *Shaker*, *Shab*, 15 *Shaw* and *Shal* subfamilies imply that they belong to a unique family of K<sup>+</sup> ion channels, called KQT-like (Wei et al., 1996). A new K<sup>+</sup> ion channel now known to be expressed in the brain raises the question of whether additional, undiscovered members of this gene family may be responsible for other forms of idiopathic, generalized epilepsies with tonic-clonic convulsions. A similar idiopathic seizure disorder seen early in development is Benign Familial Infantile Convulsions 20 (BFIC). In BFIC the seizures begin at four to eight months of age and remit after several years. BFIC maps to chromosome 19q in five Italian families (Guipponi et al., 1997). It is reasonable to hypothesize that BFIC is also caused by mutations in as yet unidentified members of the KQT-like family of K<sup>+</sup> ion channels or by minK-like proteins.

25

#### EXAMPLE 7

##### Somatic Cell Hybrid Panel Genotyping

Exploiting the putative conservation of intron-exon boundaries between *KCNQ2* and *KCNQ3* in the highly homologous transmembrane domains, a primer pair was designed from the available EST sequences (primer A: 5'-TTCCTGATTGTCCTGGGGTGCT-3' (SEQ ID NO:8), 30 primer B: 5'-TTCATCTTTGGAGCCGAGTTTGC-3' (SEQ ID NO:9)) to cross an intron. The amplified fragment contains an intron in human (1.8 kb) as well as in rodent (800 bp) genomic DNA. This primer was used to amplify the Coriell panel. The reactions were performed in a 25 µL

volume using 50 ng of template DNA and 1 unit of Taq DNA polymerase (Perkin Elmer), 10 pmol of each primer. 3 nmol of each deoxyribonucleotide in a 1.5 mM MgCl<sub>2</sub> buffer. Cycling conditions were 94°C for 4 minutes, then 30 cycles of: denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds and elongation at 72°C for 1.5 minutes, followed by a final elongation at 72°C for 10 minutes. The PCR products were electrophoresed in a 1.5% agarose gel.

#### EXAMPLE 8

##### Chromosome 8 Radiation Hybrids Panel

An HSA8 radiation hybrid panel (Lewis et al., 1995) was genotyped with specific human intronic primers (primer D: 5'-TCCATGTGGTACTCCATGTCTGAA-3' (SEQ ID NO:10), primer E: 5'-GCACGTCACATTGGGGATGTCAT-3' (SEQ ID NO:11)). The length of the PCR product is 190 bp. The reactions were performed in a 25 µL volume using 100 ng of template DNA and 1 unit of Taq polymerase (Perkin Elmer), 10 pmol of each primer, 3 nmol of each deoxyribonucleotide in a 1.5 mM MgCl<sub>2</sub> buffer. Cycling conditions were 94°C for 4 minutes, then 30 cycles of: denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds and elongation at 72°C for 30 seconds, followed by a final elongation at 72°C for 10 minutes. The PCR products were electrophoresed in a 2% agarose gel. The genotyping data was analyzed by the RHMAP V2.01 program (Boehnke et al., 1991).

#### EXAMPLE 9

##### Full Length cDNA

To identify the full length *KCNQ3* cDNA, 5' and 3' RACE were performed on adaptor-ligated fetal and adult brain cDNA (Clontech) using primers from the available EST sequences. The primers used for RACE experiments are given in Table 3. PCR products were subcloned (T/A cloning® Kit, Invitrogen) and both strands were sequenced on an ABI 377 instrument.

#### EXAMPLE 10

##### Genomic Organization/Intron-Exon Boundaries

A BAC genomic library was screened by PCR (as described for the Coriell panel) and three overlapping genomic clones were isolated. The intron/exon boundaries were identified by cloning (T/A cloning® Kit, Invitrogen) and sequencing (ABI 377) products obtained by exon-exon PCR on genomic human DNA and/or on BAC genomic clones containing the *KCNQ3* gene.

Table 3  
RACE Primers

5' RACE

5	KV1b: 5'-TGTGTTTTTGGCGTGGAGGGAGGTC-3'	(SEQ ID NO:12)
	KV2b: 5'-CAGTAACAGAAGCCAGTCTCC-3'	(SEQ ID NO:13)
	KV3b: 5'-GCAAACTCGGCTCCAAAGATGAA-3'	(SEQ ID NO:14)
	<u>KV4b: 5'-CACCAACGCGTGGTAAAGCAGC-3'</u>	<u>(SEQ ID NO:15)</u>

10

3' RACE

	KV1a: 5'-TTCCTGATTGTCCTGGGGTGCT-3'	(SEQ ID NO:16)
	<u>KV2a: 5'-AGTATCTGCCGGGCATCTCGACA-3'</u>	<u>(SEQ ID NO:17)</u>

## EXAMPLE 11

SSCP Analysis and Characterization of Mutant and Polymorphic Alleles

Sixty percent of the coding region of *KCNQ3* was amplified by PCR using primers within introns when available and analyzed by SSCP (Novex) using 20% TBE gels run at 4°C as described  
5 in Novex Thermoflow™ protocols (Novex, San Diego, CA). The PCR products presenting an SSCP polymorphism were cloned (T/A cloning® Kit, Invitrogen), nine clones were sequenced on an ABI 373 or 377 using dye-primer chemistry and analyzed with the Sequencher™ 3.0 program.

## EXAMPLE 12

Characterization of the KCNQ3 Gene

10 The KQT-like family is a recently characterized family of voltage-gated potassium channels (Wei et al., 1996). Until now, only *KCNQ2* (described in this disclosure) which is the gene mutated in the chromosome 20 BFNC disorder and *KCNQ1*, which is the chromosome 11 gene responsible for Long QT syndrome and the Jervell and Lange-Nielsen cardioauditory syndrome (Neyroud et al.,  
15 1997), were known to belong to this family. In order to identify new members of that family, possibly involved in other types of IGEs, a tBLASTx (Altschul et al., 1990) search was started with the *KCNQ2* full length cDNA against the Expressed Sequence Tags (ESTs) database. Five human EST clones were identified that presented significant homologies with *KCNQ2* (clone ID: 1-362079, 2-222324, 3-363215, 4-38822, 5-45636; Hillier et al., unpublished data). Interestingly, these clones  
20 come from two different cDNA libraries: retina (1-3) and infant brain (4-5) (Soares et al., 1994) and can be organized in two nonoverlapping contigs (1-3) and (4-5). It is demonstrated here that the two contigs belong to the same gene, *KCNQ3*.

The first step in the characterization of the new gene was genomic localization of the ESTs. Using a commercial somatic cell hybrid panel (Coriell panel (Drwinga et al., 1993)), *KCNQ3* was  
25 mapped on HSA8. In order to refine that assignment, a panel of 97 radiation hybrids previously constructed for determining the linear order and intermarker distance of chromosome 8 loci (Lewis et al., 1995) was genotyped. Specific human intronic primers were used and each RH was scored by PCR for the presence or absence of the locus. The data were analyzed using RHMAP V2.01 against results collected for other chromosome 8 markers. The retention frequency for *KCNQ3* in  
30 the RH panel was 11.7%. Tight linkage of *KCNQ3* locus was observed with markers previously mapped to chromosome band 8q24. The tightest linkage was seen with marker D8S558 (LOD 13.87,  $\theta$  of 0.047  $R_{5000}$ ). The resulting RH map is shown in Figure 5. The position of the *KCNQ3*

locus is localized to the interval defined by the markers previously linked to a chromosome 8 BFNC family (Lewis et al., 1993), making *KCNQ3* a very strong positional candidate for the chromosome 8 BFNC locus. A second Caucasian family also demonstrates suggestive linkage to the same markers (Steinlein et al., 1995).

5 A partial cDNA sequence was obtained by a series of rapid amplification of cDNA ends (RACE) experiments. 5' and 3' RACE were performed by amplifying adult and fetal brain Marathon-Ready cDNAs (Clontech) using primers derived from the two EST contigs previously identified. The primer pairs are shown in Table 3. This was used to purify a mouse genomic homolog of *KCNQ3*. After determining the mouse sequence including intron/exon junctions,  
10 primers based upon the mouse sequence were used to clone the remainder of the human cDNA for *KCNQ3*. The primers used to amplify the 5' end of the human gene were CGCGGATCATGGCATTGGAGTTC (SEQ ID NO:93) and AAGCCCCAGAGACTTCTCAGCTC (SEQ ID NO:94). The complete *KCNQ3* cDNA sequence (SEQ ID NO:6) encodes an 872 amino acid protein (SEQ ID NO:7) with six putative transmembrane domains, a pore region, a stop codon,  
15 and the 3' untranslated region containing the poly A<sup>+</sup> tail. This protein presents 58% similarity and 46% identity (calculated using BLAST) in the region from amino acid 101 to the stop codon with *KCNQ2* and is also highly conserved with *KCNQ1* (Yang et al., 1997) as well as with the *C. elegans* homologue nKQT1 (Wei et al., 1996). A comparison of sequences is shown in Figure 3. The two EST contigs are identical to amino acids 86-265 and 477-575 of *KCNQ3*, respectively (see  
20 Figure 3).

To test whether or not *KCNQ3* is the gene responsible for the chromosome 8 BFNC phenotype, mutations were looked for in one affected individual of a phenotypically well characterized three-generation Mexican-American BFNC family (Ryan et al., 1991) (see Figure 6). That family has been mapped by multipoint linkage analysis on chromosome 8q24 (Z=4.43) within  
25 the interval spanned by markers D8S198 (proximal to D8S284) and D8S274 (distal to D8S256) (see Figure 5) (Lewis et al., 1993; Dib et al., 1996). It is here shown that this chromosomal region contains the *KCNQ3* locus. So far, using intronic primers, 60% of the coding region of *KCNQ3*, containing the six transmembrane domains as well as the pore region, has been screened by a cold SSCP method. One SSCP variant was identified in a PCR fragment of 187 bp containing the  
30 transmembrane domain S5 and half of the pore. The primers used to prepare this fragment are: Ret.6a 5'-CATCACGGCCTGGTACATCGGTT-3' (SEQ ID NO:18) (corresponding to nucleotides 801-823 of SEQ ID NO:6) and Hebn2.3b 5'-AATCTCACAGAATTGGCCTCCAAG-3' (SEQ ID



NO:19). The Ret.6a primer is from coding region and the Hebn2.3b primer is from intronic region. This SSCP variant is in perfect cosegregation with the BFNC phenotype and it is also present in a single non-penetrant individual carrying the disease-marker haplotype (Figure 6). Furthermore, this SSCP variant is absent from a panel of 72 Caucasian and 60 Mexican-American (264 chromosomes) unrelated individuals used as the control group. To characterize the nucleotide change of this variant, the PCR product of one affected individual was cloned and nine clones were sequenced on both strands. Four clones contained the wild-type allele and five the mutated allele. The mutation is a single missense mutation Gly (GGC) to Val (GTC) in position 310 of the highly conserved pore region (the mutation occurring at base 947 of SEQ ID NO:6). In addition, a silent polymorphism (frequency of 0.4%) was found in one Mexican-American control in the transmembrane region S5 at L278 (CTT - CTC) (the polymorphism is at base 852 of SEQ ID NO:6). Four other polymorphisms in KCNQ3 have been seen. These are at N220 (AAC or AAT), Gly244 (GGT or GGC), L357 (CTG or CTC) and I860 (ATT or ATC). These polymorphisms are at base numbers 678, 750, 1089 and 2598 of SEQ ID NO:6, respectively.

15 In addition, some individual probands with juvenile myoclonic epilepsy were screened with SSCP. JME is an inherited childhood seizure disorder. KCNQ3 was mutated in one individual who was tested. The mutation was found in an alternatively spliced exon that lies in an intron which splits codon 412. This alternatively spliced exon was found in adult brain after RACE experiments. This exon is SEQ ID NO:92. The exon was seen in an adult brain cDNA clone obtained from Clontech. This exon is 130 nucleotides long which is not a multiple of 3. Therefore the presence of this exon results not only in the addition of extra amino acid sequence but causes a frameshift (1 extra base) which results in a stop codon within the normal coding region of the gene. The mutation found in the JME proband is a 1 base pair deletion in the alternatively spliced exon (the loss of the G at base 118 of SEQ ID NO:92) that results in the frameshift from the alternative exon going back into frame resulting in a KCNQ3 with an additional 43 amino acid residues between amino acid residues 412 and 413 of the wild-type, and thus alters the protein in the brain cells of the JME proband. The patient with this deletion has a mother who has epilepsy, however this particular mutation is from the father, not from the mother. JME is a common, inherited childhood epilepsy and most likely is caused by defects not only in KCNQ3 but also in other genes.

30 This finding brings to three the number of human members of the KQT-like family, two of which are expressed in brain and one in heart. Defects in all three K<sup>+</sup> channel genes cause human diseases associated with altered regulation of excitability. Taking all these findings together, there

is strong evidence that *KCNQ2* and *KCNQ3*, as well as undiscovered genes of the same family or genes belonging to the same pathway, are involved in IGEs. Screening these KQT-like K<sup>+</sup> channel genes as well as other K<sup>+</sup> channel genes belonging to different families (Wei et al., 1996) for mutations in individuals with common types of IGEs will be a powerful alternative for identifying disease-causing genes. This is especially true given the difficult and controversial tentative linkages described in IGE disease pedigrees (Leppert et al., 1993).

### EXAMPLE 13

#### Generation of Polyclonal Antibody against *KCNQ2* or *KCNQ3*

10 Segments of *KCNQ2* or *KCNQ3* coding sequence are expressed as fusion protein in *E. coli*. The overexpressed protein is purified by gel elution and used to immunize rabbits and mice using a procedure similar to the one described by Harlow and Lane, 1988. This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

Briefly, a stretch of *KCNQ2* or *KCNQ3* coding sequence is cloned as a fusion protein in  
15 plasmid PET5A (Novagen, Inc., Madison, WI). After induction with IPTG, the overexpression of a fusion protein with the expected molecular weight is verified by SDS/PAGE. Fusion protein is purified from the gel by electroelution. Identification of the protein as the *KCNQ2* or *KCNQ3* fusion product is verified by protein sequencing at the N-terminus. Next, the purified protein is used as immunogen in rabbits. Rabbits are immunized with 100 µg of the protein in complete Freund's  
20 adjuvant and boosted twice in 3 week intervals, first with 100 µg of immunogen in incomplete Freund's adjuvant followed by 100 µg of immunogen in PBS. Antibody containing serum is collected two weeks thereafter.

This procedure is repeated to generate antibodies against the mutant forms of the *KCNQ2* or *KCNQ3* gene product. These antibodies, in conjunction with antibodies to wild type *KCNQ2* or  
25 *KCNQ3*, are used to detect the presence and the relative level of the mutant forms in various tissues and biological fluids.

### EXAMPLE 14

#### Generation of Monoclonal Antibodies Specific for *KCNQ2* or *KCNQ3*

30 Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising intact *KCNQ2*, intact *KCNQ3*, *KCNQ2* peptides or

KCNQ3 peptides (wild type or mutant) conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known.

The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100  $\mu$ g of immunogen and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

Spleens are removed from immune mice and a single cell suspension is prepared (see Harlow and Lane, 1988). Cell fusions are performed essentially as described by Kohler and Milstein, 1975. Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) are fused with immune spleen cells using polyethylene glycol as described by Harlow and Lane, 1988. Cells are plated at a density of  $2 \times 10^5$  cells/well in 96 well tissue culture plates. Individual wells are examined for growth and the supernatants of wells with growth are tested for the presence of KCNQ2 or KCNQ3 specific antibodies by ELISA or RIA using wild type or mutant KCNQ2 or KCNQ3 target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibody for characterization and assay development.

#### EXAMPLE 15

##### Sandwich Assay for KCNQ2 or KCNQ3

Monoclonal antibody is attached to a solid surface such as a plate, tube, bead or particle. Preferably, the antibody is attached to the well surface of a 96-well ELISA plate. 100  $\mu$ L sample (e.g., serum, urine, tissue cytosol) containing the KCNQ2 or KCNQ3 peptide/protein (wild-type or mutants) is added to the solid phase antibody. The sample is incubated for 2 hrs at room temperature. Next the sample fluid is decanted, and the solid phase is washed with buffer to remove unbound material. 100  $\mu$ L of a second monoclonal antibody (to a different determinant on the KCNQ2 or KCNQ3 peptide/protein) is added to the solid phase. This antibody is labeled with a detector molecule (e.g.,  $^{125}$ I, enzyme, fluorophore, or a chromophore) and the solid phase with the second antibody is incubated for two hrs at room temperature. The second antibody is decanted and the solid phase is washed with buffer to remove unbound material.

The amount of bound label, which is proportional to the amount of KCNQ2 or KCNQ3 peptide/protein present in the sample, is quantified. Separate assays are performed using monoclonal antibodies which are specific for the wild-type KCNQ2 or KCNQ3 as well as monoclonal antibodies specific for each of the mutations identified in KCNQ2 or KCNQ3.

5

#### EXAMPLE 16

##### Assay to Screen Drugs Affecting the KCNQ2 or KCNQ3 K<sup>+</sup> Channel

With the knowledge that KCNQ2 and KCNQ3 each forms a potassium channel, it is now possible to devise an assay to screen for drugs which will have an effect on one or both of these  
10 channels. The gene is transfected into oocytes or mammalian cells and expressed as described above. When the gene used for transfection contains a mutation which causes BFNC, rolandic epilepsy or JME, a change in the induced current is seen as compared to transfection with wild-type gene only. A drug candidate is added to the bathing solution of the transfected cells to test the effects of the drug candidates upon the induced current. A drug candidate which alters the induced  
15 current such that it is closer to the current seen with cells cotransfected with wild-type *KCNQ2* or wild-type *KCNQ3* is useful for treating BFNC, rolandic epilepsy or JME.

#### EXAMPLE 17

##### PRIMER PAIRS FOR SCREENING EACH EXON OF *KCNQ2* FOR MUTATION

20 The genomic *KCNQ2* has been sequenced in the intron/exon borders and primer pairs useful for amplifying each exon have been developed. These primer pairs are shown in Table 4. For exons 13 and 17 primers within the exons are also utilized. Some exon/intron sequence is shown in Figures 7A-O.

#### EXAMPLE 18

##### INTRON SEQUENCE OF *KCNQ3* AND 25 PRIMER PAIRS FOR AMPLIFYING THE EXONS OF *KCNQ3*

Although the complete cDNA for *KCNQ3* has been obtained and sequenced, the complete genomic DNA has not yet been sequenced. However, much of the intron DNA has been sequenced and this sequence information has been utilized to develop primer pairs which are useful for  
30 amplifying each exon. The intron/exon sequence is shown in Figures 8A-O. Some useful primer pairs for amplifying each exon are shown in Table 5 although one of skill in the art can easily develop other primer pairs using the intron sequence shown in Figures 8A-O.

Table 4

Exon	Domain	Primer Sequence (SEQ ID NO:)
1	met + SI	
2	SI + SII	TTCCTCCTGGTTTTCTCCTGCCT (SEQ ID NO:22) AAGACAGACGCCCAGGCAGCT (SEQ ID NO:23)
5 3	SII + SIII	AGGCCTCAAGGTGGCCTCAGCTTT (SEQ ID NO:24) CTGGCCCTGATTCTAGCAATAC (SEQ ID NO:25)
4	SIII + SIV	ACATCATGGTGCTCATCGCCTCC (SEQ ID NO:97) TGTGGGCATAGACCACAGAGCC (SEQ ID NO:26)
5	SV + pore	TGGTCACTGCCTGGTACATCGG (SEQ ID NO:27) ATGGAGCAGGCTCAGCCAGTGAGA (SEQ ID NO:28)
6	pore + SVI	GCAGGCCCTTCGTGTGACTAGA (SEQ ID NO:29) ACCTAGGGAAGTGTGCCAGG (SEQ ID NO:30)
7	SVI	ATGGTCTGACCCTGATGAATTGG (SEQ ID NO:31) GCGGCCTCCACTCCTCAACAA (SEQ ID NO:32)
10 8	C-term	
9	C-term	
10	variable	CCGCCGGGCACCTGCCACCAA (SEQ ID NO:33) GCTTGACAGCTCCATGGGCAG (SEQ ID NO:34)
11	C-term	GCTGTGCAAGCAGAGGGAGGTG (SEQ ID NO:98) CTGTCCTGGCGTGTCTTCTGTG (SEQ ID NO:99)
12	variable cysteine insertion	CCCAGGACTAACTGTGCTCTCC (SEQ ID NO:35) CCGTGCAGCAGCCGTCAGTCC (SEQ ID NO:36)
15 13	C-term	GCAGAGTGACTTCTCTCCCTGTT (SEQ ID NO:37) GTCCCCGAAGCTCCAGCTCTT (SEQ ID NO:38)  AAGATCGTGTCTTCTCCAGCCC (SEQ ID NO:39) GATGGACCAGGAGAGGATGCGG (SEQ ID NO:40)
14	C-term	CCCTCACGGCATGTGTCCTTCC (SEQ ID NO:41) AGCGGGAGGCCCTCCTCACT (SEQ ID NO:42)
15	C-term	GGTCTCTGGCCCAGGGCTCACA (SEQ ID NO:43) CTTGTCCTCTGCTGGACAGGCA (SEQ ID NO:44)
16	C-term	TTGACGGCAGGCACACAGCC (SEQ ID NO:45)

Exon	Domain	Primer Sequence (SEQ ID NO:)
17	C-term	CCCAGCCCAGCAGCCCCTTTT (SEQ ID NO:46) AGGTGGAGGGCGGACACTGGA (SEQ ID NO:47)  CTCCACGGGCCAGAAGAACTTC (SEQ ID NO:48) GATGGAGATGGACGTGTCGCTGT (SEQ ID NO:49)  TGGAGTTCCTGCGGCAGGAGGACAC (SEQ ID NO:50) GGTGTCTGACTCTCCCTCCGCAA (SEQ ID NO:51)  GTGGCGCCTTGTGCCAAAGTCA (SEQ ID NO:52) ACCTCGGAGGCACCGTGCTGA (SEQ ID NO:53)

Table 5

Pair	Sequence 5'→3' (SEQ ID NO:)	Size	Temp	Part of the gene
1	GCGACGTGGAGCAAGTACCTTG (54) CACCAACGCGTGGTAAAGCAGC (55)	245	62	before S1
2	ATGACTCAAAGGTTCTTAGTCCA (56) GAAGCCCAACCAGAAGCATTAC (57)	174	62	S1 to beginning of S2
5 3	TCAGTGCCTCTCCATATGCTCTT (58) ACTGAGGAGGCTGGGAGGCTC (59)	194	62	end of S2 to beginning of S3
4	GATGACGCCATTGCTTTCGCATGA (60) GTGGGAAGCCCATGTGGTCCTG (61)	298	65	end of S3 to S4
5	CATCCACTCAACGACTCCCCAG (62) AATCTCACAGAATTGGCCTCCAAG (63)	249	65	S5 to beginning of the pore
6	TCCATGTGGTACTCCATGTCTGAA (64) GCACGTCACATTGGGGATGTCAT (65)	190	58	end of the pore to beginning of S6
7	GGAATGCTGGGACAGTCTAGCTG (66) TACATATGCATGGATCTTAATCCCAT (67)	203	58	end of S6 to start of C-terminal part
10 8	AAAGTTTCAGGTGGTGCCCACTCA (68) GAGGCCACAGACACGAATACAGAC (69)	230	65	C- terminal
9	TGGGTAAACCCGCCTCCTTCATTG (70) ACTCTATCTTGGGACCAGCATGAC (71)	306	65	C- terminal
10 10	TAAGAGCCTGCACTGAAGGAGGA (72) GGGGAGGAAGAAGTGGAAGAGAC (73)	302	65	C- terminal
11	CAGGTCTGTGGCCTGCCGTTTCAT (74) CCTTCCTGTGGGAGTTGAGCTGG (75)	233	65	C- terminal
12	GTTTGCTAGCCTTCTGTTATAGCT (76) GGGAGCGCAGTCCCTCCAGAT (77)	239	62	C- terminal
15 13	CTTATATATTCCAAACCCTTATCTCA (78) GGTGGGGATCGTTGCTATTGGTT (79)	277	62	C- terminal
14	AACCAATAGCAACGATCCCCACC (80) CTGACTTTGTCAATGGTCACCTGG (81)	303	65	C- terminal after last intron
15	CGGAACCACCCTACAGCTTCCA (82) GGGAGTGGCAGCTCACTCGGGA (83)	210	65	C- terminal after last intron
16	AGGCCACGGTCCTGCCTATCT (84) CCATTGGGGCCGAACACATAATC (85)	236	65	C- terminal after last intron
17	CTTCAGCATCTCCAGGACAGAG (86) AAGGAGGGGTCAGCCAGTGACCT (87)	228	65	C- terminal after the STOP codon

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended

5 claims.



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WHAT IS CLAIMED IS:

1. An isolated nucleic acid or its complement comprising nucleic acid encoding a protein selected from SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:89, SEQ ID NO:91, or SEQ ID NO:96.
2. An isolated nucleic acid or its complement according to claim 1 wherein said nucleic acid comprises a nucleic acid with a sequence of nucleotides 128-2743 of SEQ ID NO:1, nucleotides 19-2634 of SEQ ID NO:6, nucleotides 1-2273 of SEQ ID NO:88, nucleotides 202-2812 of SEQ ID NO:90, or nucleotides 128-2917 of SEQ ID NO:95.
3. An isolated nucleic acid or its complement comprising nucleic acid coding for a mutant human KCNQ2 or KCNQ3 polypeptide which causes benign familial neonatal convulsions (BFNC), juvenile myoclonic epilepsy (JME) or rolandic epilepsy.
4. An isolated nucleic acid according to claim 3 wherein said isolated nucleic acid comprises a mutation which causes BFNC, JME, or rolandic epilepsy wherein said mutation is selected from the group consisting of: a G at nucleotide 978 of SEQ ID NO:1, an A at nucleotide 1043 of SEQ ID NO:1, a T at nucleotide 1094 of SEQ ID NO:1, an A at nucleotide 1125 of SEQ ID NO:1, a T at nucleotide 1469 of SEQ ID NO:1, an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides after nucleotide 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts codon 544 of KCNQ2, a mutation causing a stop codon at or before codon 319 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 524 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 323 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 448 of SEQ ID NO:2, a T at nucleotide 947 of SEQ ID NO:6, and the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
5. An isolated nucleic acid according to claim 3 wherein said isolated nucleic acid encodes a cysteine at codon 284 of SEQ ID NO:2, a threonine at codon 306 of SEQ ID NO:2, a glutamine at codon 333 of SEQ ID NO:2 or a valine at codon 310 of SEQ ID NO:7.

6. A nucleic acid probe which hybridizes specifically to the nucleic acid of claim 1 under high stringency.
7. A nucleic acid probe which hybridizes specifically to a nucleic acid of claim 3 under stringent hybridization conditions wherein said stringent hybridization conditions prevent said nucleic acid probe from hybridizing to nucleic acid defined by SEQ ID NO:1 or SEQ ID NO:6.
8. A nucleic acid probe which hybridizes specifically to a nucleic acid of claim 4 under stringent hybridization conditions wherein said stringent hybridization conditions prevent said nucleic acid probe from hybridizing to nucleic acid defined by SEQ ID NO:1 or SEQ ID NO:6.
9. A method for diagnosing a mutation which causes BFNC, JME, or rolandic epilepsy wherein said method comprises hybridizing a probe of claim 7 to a patient's sample of DNA or RNA under stringent conditions which allow hybridization of said probe to nucleic acid comprising said mutation but prevent hybridization of said probe to wild-type human *KCNQ2* or *KCNQ3* wherein the presence of a hybridization signal indicates the presence of said mutation.
10. A method for diagnosing a mutation which causes BFNC, JME, or rolandic epilepsy wherein said method comprises hybridizing a probe of claim 8 to a patient's sample of DNA or RNA under stringent conditions which allow hybridization of said probe to nucleic acid comprising said mutation but prevent hybridization of said probe to wild-type human *KCNQ2* or *KCNQ3* wherein the presence of a hybridization signal indicates the presence of said mutation.
11. A method according to claim 9 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized.
12. A method according to claim 10 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized.

13. A method according to claim 9 wherein hybridization is performed *in situ*.
14. A method according to claim 10 wherein hybridization is performed *in situ*.
15. A method for diagnosing the presence of a mutation in human *KCNQ2* or *KCNQ3* which causes BFNC, JME, or rolandic epilepsy wherein said method is performed by means which identify the presence of said mutation.
16. The method of claim 15 wherein said mutation is the presence of a G at nucleotide number 978 of SEQ ID NO:1, an A at nucleotide number 1043 of SEQ ID NO:1, a T at nucleotide number 1094 of SEQ ID NO:1, an A at nucleotide number 1125 of SEQ ID NO:1, a T at nucleotide 1469 of SEQ ID NO:1, an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides after nucleotide 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts codon 544 of SEQ ID NO:2, a mutation resulting in a stop codon at or before codon 319 of SEQ ID NO:2, a mutation resulting in a stop codon at or before codon 524 of SEQ ID NO:2, a mutation resulting in a stop codon at or before codon 323 of SEQ ID NO:2, a mutation resulting in a stop codon at or before codon 448 of SEQ ID NO:2, a T at nucleotide 947 of SEQ ID NO:6, or the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
17. The method of claim 15 wherein said means comprises using a single-stranded conformation polymorphism technique to assay for said mutation.
18. The method of claim 17 wherein said mutation is the presence of a G at nucleotide number 978 of SEQ ID NO:1, an A at nucleotide number 1043 of SEQ ID NO:1, a T at nucleotide number 1094 of SEQ ID NO:1, an A at nucleotide number 1125 of SEQ ID NO:1, a T at nucleotide number 1469 of SEQ ID NO:1, an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides following nucleotide number 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts

codon 544 of *KCNQ2*, a mutation causing a stop codon at or before codon 319 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 524 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 323 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 448 of SEQ ID NO:2, a T at 947 of SEQ ID NO:6, or the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.

19. The method of claim 18 wherein said mutation is a T at 947 of SEQ ID NO:6 and further wherein said single-stranded conformation polymorphism technique uses amplified nucleic acid wherein said amplified nucleic acid was prepared using primers of SEQ ID NO:18 and SEQ ID NO:19.
20. The method of claim 15 wherein said means comprises sequencing human *KCNQ2* or *KCNQ3*.
21. The method of claim 20 wherein said mutation is the presence of a G at nucleotide number 978 of SEQ ID NO:1, an A at nucleotide number 1043 of SEQ ID NO:1, a T at nucleotide 1094 of SEQ ID NO:1, an A at nucleotide 1125, a T at nucleotide 1469 of SEQ ID NO:1, an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides after nucleotide 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts codon 544 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 319 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 524 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 323 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 448 of SEQ ID NO:2, a T at nucleotide 947 of SEQ ID NO:6, or the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
22. The method of claim 15 wherein said means comprises performing an RNase assay.
23. The method of claim 22 wherein said mutation is the presence of a G at nucleotide number 978 of SEQ ID NO:1, an A at nucleotide number 1043 of SEQ ID NO:1, a T at nucleotide 1094 of SEQ ID NO:1, an A at nucleotide 1125, a T at nucleotide 1469 of SEQ ID NO:1,

an insertion of two nucleotides between nucleotides 975 and 976 of SEQ ID NO:1, an insertion of 5 nucleotides after nucleotide 2736 of SEQ ID NO:1, a deletion of 13 nucleotides consisting of nucleotides 1691-1703 of SEQ ID NO:1, an A rather than a G at the 3' end of the intron which interrupts codon 544 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 319 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 524 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 323 of SEQ ID NO:2, a mutation causing a stop codon at or before codon 448 of SEQ ID NO:2, a T at nucleotide 947 of SEQ ID NO:6, or the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.

24. An antibody which binds to a polypeptide of SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:89, or SEQ ID NO:91.
25. An antibody which binds to a mutant human KCNQ2 or mutant human KCNQ3 polypeptide but not to wild-type human KCNQ2 or wild-type human KCNQ3 polypeptide, wherein said mutant polypeptide causes BFNC, JME or rolandic epilepsy.
26. The antibody of claim 25 wherein said mutant polypeptide comprises a cysteine at amino acid residue 284 of SEQ ID NO:2, a threonine at amino acid residue 306 of SEQ ID NO:2, a glutamine at amino acid residue 333 of SEQ ID NO:2, a valine at amino acid residue 310 of SEQ ID NO:7, or wherein said mutant polypeptide is SEQ ID NO:96.
27. A method for diagnosing BFNC, JME, or rolandic epilepsy in a human patient, said method comprising an assay for the presence of mutant KCNQ2 or mutant KCNQ3 polypeptide in said patient by reacting a sample comprising protein from said patient with an antibody of claim 25 wherein the presence of a positive reaction is indicative of BFNC, JME, or rolandic epilepsy.
28. The method of claim 27 wherein said mutant KCNQ2 or mutant KCNQ3 is selected from the group consisting of (a) a KCNQ2 comprising a cysteine at amino acid residue 284 of SEQ ID NO:2, (b) a KCNQ2 comprising a threonine at amino acid residue 306 of SEQ ID NO:2, (c) a KCNQ2 comprising a glutamine at amino acid residue 333 of SEQ ID NO:2, (d)



a KCNQ2 encoded by a DNA of mutated SEQ ID NO:1 wherein said mutated SEQ ID NO:1 is SEQ ID NO:1 altered by an insertion of a GT between nucleotides 975 and 976, (e) a KCNQ2 encoded by a DNA of mutated SEQ ID NO:1 wherein said mutated SEQ ID NO:1 is SEQ ID NO:1 altered by an insertion of a GGGCC following nucleotide 2736 of SEQ ID NO:1, (f) a KCNQ2 encoded by a DNA of mutated SEQ ID NO:1 wherein said mutated SEQ ID NO:1 is SEQ ID NO:1 altered by the deletion of 13 nucleotides consisting of nucleotides 1691-1703, (g) a KCNQ2 comprising amino acid residues 1-318 of SEQ ID NO:2, (h) a KCNQ2 comprising amino acid residues 1-523 of SEQ ID NO:2, (i) a KCNQ2 comprising amino acid residues 1-322 of SEQ ID NO:2, (j) a KCNQ2 comprising amino acid residues 1-448 of SEQ ID NO:2, and (k) a KCNQ3 comprising a valine at amino acid residue 310 of SEQ ID NO:7.

29. The method of claim 27 wherein said antibody is a monoclonal antibody..
30. The method of claim 28 wherein said antibody is a monoclonal antibody.
31. An isolated human KCNQ2 or KCNQ3 polypeptide comprising a mutation which causes BFNC, JME, or rolandic epilepsy.
32. The polypeptide of claim 31 wherein said mutation is a cysteine at amino acid residue 284 of SEQ ID NO:2, a threonine at amino acid residue 306 of SEQ ID NO:2, a glutamine at amino acid residue 333 of SEQ ID NO:2, or a valine at amino acid residue 310 of SEQ ID NO:7.
33. An isolated KCNQ2 polypeptide selected from the group consisting of (a) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a GT insertion between bases 975 and 976, (b) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a 13 base deletion consisting of nucleotides 1691-1703, (c) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a GGGCC insertion following nucleotide 2736, (d) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a T at nucleotide 1094, and (e) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a T at nucleotide 1469.

34. A method for diagnosing BFNC, JME, or rolandic epilepsy in a person wherein said method comprises sequencing KCNQ2 or KCNQ3 polypeptide from said person or sequencing KCNQ2 or KCNQ3 polypeptide synthesized from nucleic acid derived from said person wherein the presence of a cysteine at amino acid residue 284 of KCNQ2, a threonine at amino acid residue 306 of KCNQ2, a glutamine at amino acid residue 333 of KCNQ2, or a valine at amino acid residue 310 of KCNQ3 is indicative of BFNC, JME or rolandic epilepsy.
35. A method for diagnosing BFNC, JME, or rolandic epilepsy in a person wherein said method comprises sequencing KCNQ2 polypeptide from said person or sequencing KCNQ2 polypeptide synthesized from nucleic acid derived from said person wherein the presence of (a) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a GT insertion between bases 975 and 976, (b) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a 13 base deletion consisting of nucleotides 1691-1703, (c) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a GGGCC insertion following nucleotide 2736, (d) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a T at nucleotide 1094, and (e) a polypeptide encoded by a DNA wherein said DNA is SEQ ID NO:1 with a T at nucleotide 1469 is indicative of BFNC, JME or rolandic epilepsy.
36. A cell transfected with the DNA of claim 1.
37. A cell transfected with the DNA of claim 2.
38. A cell transfected with the DNA of claim 3.
39. A method to screen for drugs which are useful in treating or preventing BFNC, JME or rolandic epilepsy, said method comprising:
- a) preparing cells with wild-type *KCNQ2* or wild-type *KCNQ3*;
  - b) placing the cells of step (a) into a bathing solution to measure current;
  - c) measuring an induced  $K^+$  current in the cells of step (b);

d) preparing cells with mutant *KCNQ2* if *KCNQ2* is used in step (a) or preparing cells with mutant *KCNQ3* if *KCNQ3* is used in step (a);  
e) placing the cells of step (d) into a bathing solution to measure current;  
f) measuring an induced K<sup>+</sup> current in the cells of step (e);  
g) adding a drug to the bathing solution of step (e);  
h) measuring an induced K<sup>+</sup> current in the cells of step (g); and  
i) determining whether the drug resulted in an induced K<sup>+</sup> current more similar to or less similar to the induced K<sup>+</sup> current seen in cells with wild-type *KCNQ2* or *KCNQ3* as compared to the current seen in cells with mutant *KCNQ2* or mutant *KCNQ3* in the absence of said drug,  
wherein a drug which results in a current more similar to the current seen in cells with wild-type *KCNQ2* or wild-type *KCNQ3* is useful in treating or preventing BFNC, JME or rolandic epilepsy.

40. The method of claim 38 wherein said mutant *KCNQ2* comprises a mutation shown in Table 2, said mutant *KCNQ3* comprises a T at nucleotide 947 of SEQ ID NO:6, or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
41. The method of claim 39 wherein said cells are mammalian.
42. The method of claim 40 wherein said cells are mammalian.
43. The method of claim 39 wherein said cells are CHO cells.
44. The method of claim 40 wherein said cells are CHO cells.
45. The method according to claim 39 wherein human *KCNQ2* RNA or human *KCNQ3* RNA is used in a transfection step.
46. A nucleic acid vector comprising wild-type human *KCNQ2* or *KCNQ3*.
47. A nucleic acid vector comprising mutant human *KCNQ2* or *KCNQ3*.

48. The nucleic acid vector of claim 47 wherein said mutant human *KCNQ2* comprises a mutation shown in Table 2, wherein said mutant human *KCNQ3* comprises a T at the nucleotide represented by nucleotide 947 of SEQ ID NO:6, or wherein said mutant human *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
49. A nonhuman, transgenic animal wherein said animal comprises wild-type human *KCNQ2* or wild-type human *KCNQ3*.
50. A nonhuman, transgenic animal wherein said animal comprises mutant human *KCNQ2* or mutant human *KCNQ3*.
51. The animal of claim 56 wherein said mutant human *KCNQ2* comprises a mutation shown in Table 2, said mutant *KCNQ3* comprises a T at nucleotide 947 of SEQ ID NO:6, or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
52. A method to screen for drugs which are useful in treating or preventing BFNC, JME or rolandic epilepsy, said method comprising:
- a) preparing a transgenic animal with wild-type human *KCNQ2* or wild-type human *KCNQ3*;
  - b) measuring an induced  $K^+$  current in the transgenic animal of step (a);
  - c) preparing a transgenic animal with mutant human *KCNQ2* if *KCNQ2* is used in step (a) or with mutant human *KCNQ3* if *KCNQ3* is used in step (a);
  - d) measuring an induced  $K^+$  current in the transgenic animal of step (c);
  - e) administering a drug to the transgenic animal of step (c);
  - f) measuring an induced  $K^+$  current in the drug-treated animal of step (e);
  - g) determining whether the drug resulted in an induced  $K^+$  current more similar to or less similar to the induced  $K^+$  current seen in the transgenic animal with wild-type human *KCNQ2* or wild-type human *KCNQ3* as compared to the current seen in a transgenic animal with mutant human *KCNQ2* or mutant human *KCNQ3* in the absence of said drug,

wherein a drug which results in a current more similar to the current seen in transgenic animals with wild-type human *KCNQ2* or wild-type human *KCNQ3* is useful in treating or preventing BFNC, JME or rolandic epilepsy.

53. The method of claim 52 wherein said mutant human *KCNQ2* comprises a mutation shown in Table 2, said mutant *KCNQ3* comprises a T at nucleotide 947 of SEQ ID NO:6, or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
54. A method for diagnosing a mutation which causes BFNC, JME or rolandic epilepsy comprising sequencing *KCNQ2* or *KCNQ3* in a patient's sample of DNA to determine the presence or absence of mutations which cause BFNC, JME or rolandic epilepsy.
55. The method of claim 54 wherein said mutations are selected from the mutations shown in Table 2, from the presence of a T at nucleotide 947 in SEQ ID NO:6 or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
56. The method of claim 54 wherein said patient's sample of DNA has been amplified.
57. The method of claim 55 wherein said patient's sample of DNA has been amplified.
58. A method for diagnosing a mutation which causes BFNC, JME or rolandic epilepsy wherein said method comprises sequencing a *KCNQ2* gene or a *KCNQ3* gene in a patient's sample of RNA to determine the presence or absence of mutations which cause BFNC, JME or rolandic epilepsy.
59. The method of claim 58 wherein said mutations are selected from the mutations shown in Table 2, the presence of a T at nucleotide 947 of SEQ ID NO:6, or said mutant *KCNQ3* comprises the presence of alternative exon of SEQ ID NO:92 in mRNA of *KCNQ3*.
60. A method for diagnosing a mutation which causes BFNC, JME or rolandic epilepsy wherein said method comprises determining *KCNQ2* or *KCNQ3* sequence in a patient by preparing

cDNA from RNA taken from said patient and sequencing said cDNA to determine the presence or absence of mutations which cause BFNC, JME or rolandic epilepsy.

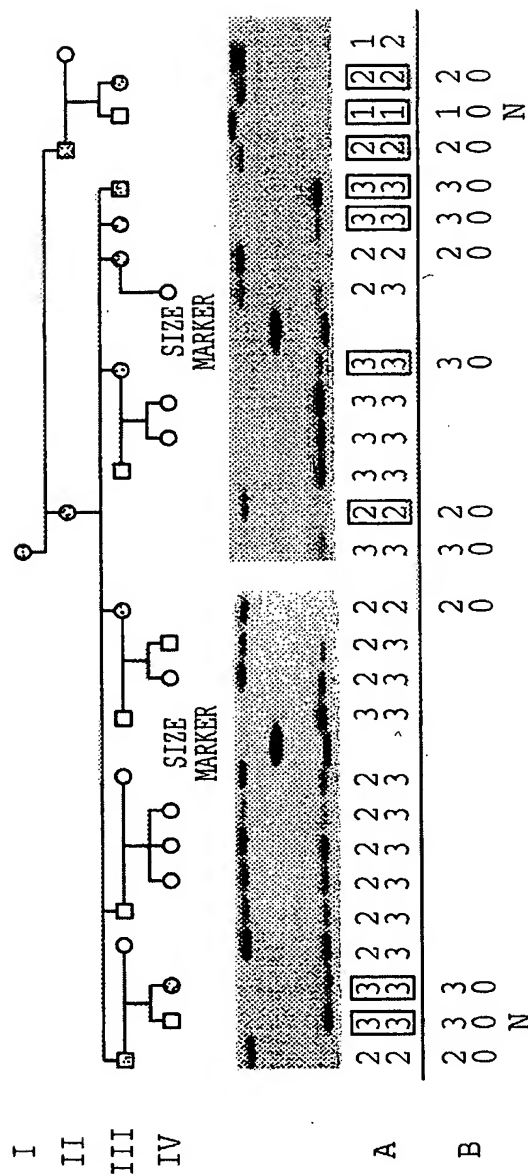
61. A method of diagnosing the presence of BFNC, JME or rolandic epilepsy by performing in situ hybridization with a probe specific for *KCNQ2* or *KCNQ3* wherein the presence of only a single copy of either *KCNQ2* or *KCNQ3* indicates the presence of BFNC, JME or rolandic epilepsy.
62. A pair of single-stranded DNA primers for determination of a nucleotide sequence of *KCNQ2* by a polymerase chain reaction, the sequence of said primers being derived from human chromosome 20q13, wherein the use of said primers in a polymerase chain reaction results in the synthesis of DNA having all or part of the sequence of *KCNQ2*.
63. A pair of single-stranded DNA primers of claim 62 wherein said pair is selected from:
  - (a) SEQ ID NO:22 and SEQ ID NO:23,
  - (b) SEQ ID NO:24 and SEQ ID NO:25,
  - (c) SEQ ID NO:27 and SEQ ID NO:28,
  - (d) SEQ ID NO:29 and SEQ ID NO:30,
  - (e) SEQ ID NO:31 and SEQ ID NO:32,
  - (f) SEQ ID NO:33 and SEQ ID NO:34,
  - (g) SEQ ID NO:35 and SEQ ID NO:36,
  - (h) SEQ ID NO:37 and SEQ ID NO:38,
  - (i) SEQ ID NO:39 and SEQ ID NO:40,
  - (j) SEQ ID NO:41 and SEQ ID NO:42,
  - (k) SEQ ID NO:43 and SEQ ID NO:44,
  - (l) SEQ ID NO:46 and SEQ ID NO:47,
  - (m) SEQ ID NO:48 and SEQ ID NO:49,
  - (n) SEQ ID NO:50 and SEQ ID NO:51, or
  - (o) SEQ ID NO:52 and SEQ ID NO:53.
64. A pair of single-stranded DNA primers for determination of a nucleotide sequence of *KCNQ3* by a polymerase chain reaction, the sequence of said primers being derived from

human chromosome 8q24, wherein the use of said primers in a polymerase chain reaction results in the synthesis of DNA having all or part of the sequence of *KCNQ3*.

65. A pair of single-stranded DNA primers of claim 64 wherein said pair is selected from:
- (a) SEQ ID NO:54 and SEQ ID NO:55,
  - (b) SEQ ID NO:56 and SEQ ID NO:57,
  - (c) SEQ ID NO:58 and SEQ ID NO:59,
  - (d) SEQ ID NO:60 and SEQ ID NO:61,
  - (e) SEQ ID NO:62 and SEQ ID NO:63,
  - (f) SEQ ID NO:64 and SEQ ID NO:65,
  - (g) SEQ ID NO:66 and SEQ ID NO:67,
  - (h) SEQ ID NO:68 and SEQ ID NO:69,
  - (i) SEQ ID NO:70 and SEQ ID NO:71,
  - (j) SEQ ID NO:72 and SEQ ID NO:73,
  - (k) SEQ ID NO:74 and SEQ ID NO:75,
  - (l) SEQ ID NO:76 and SEQ ID NO:77,
  - (m) SEQ ID NO:78 and SEQ ID NO:79,
  - (n) SEQ ID NO:80 and SEQ ID NO:81,
  - (o) SEQ ID NO:82 and SEQ ID NO:83,
  - (p) SEQ ID NO:84 and SEQ ID NO:85, or
  - (q) SEQ ID NO:86 and SEQ ID NO:87.
66. An isolated DNA comprising DNA having at least 8 consecutive nucleotides of bases 1244-3232 of SEQ ID NO:1 or at least 8 consecutive nucleotides of SEQ ID NO:6.
67. The isolated DNA of claim 66 wherein said DNA comprises at least 15 consecutive nucleotides of bases 1244-3232 of SEQ ID NO:1 or at least 15 consecutive nucleotides of SEQ ID NO:6.
68. An isolated DNA comprising DNA having at least 8 consecutive nucleotides of any one of SEQ ID NOs:100-129.

69. An isolated DNA comprising DNA having at least 15 consecutive nucleotides of any one of SEQ ID NOs:100-129.
70. An isolated nucleic acid comprising a sequence selected from any one of SEQ ID NOs:100-129.

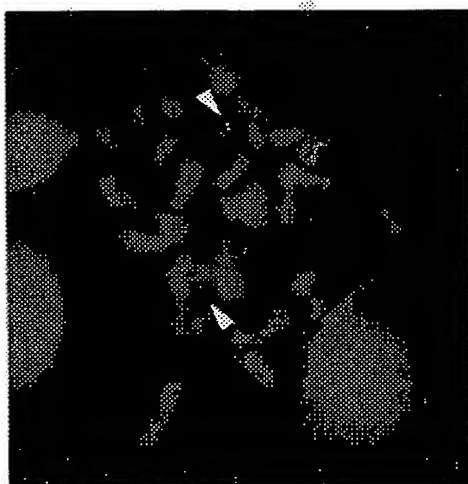






D20S24

FIG. 2A



P1-K09-6b

FIG. 2B



P1-K09-7

FIG. 2C

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KCNQ3	1	MGLKARRAAGAGGGGGGGAANPAGDAAAAGDEERKVG LAPDVEQVT	54
KCNQ2		.....MVQSRNGGVYGPSPGEKKLVGF	24
KCNQ1		MAAASSPPRAERKRWGRLPGARRGSAGLAKC PPSLEAEGPAGGALYAP	
nQT1		MDEESGSSVSMULTMRKLSPVAMVSRSQKTTDQAAPSDEQQEAGSSSAIGQESR	
		S1	
KCNQ3		LALGAGADKDTLLLEGGRDEGQRTTQIGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERPRGW. ALLYHALVFLVLVGLCLILAVLT	143
KCNQ2		VGLDPGAPDSTRDGA LL IAGSEAPKRGSI LSKPRAGGAGAGKPPKRNAFYRK LQNF LYNVLERPRGW. AFYHAYVFLVFLVFSCLVLSVFS	113
KCNQ1		IAPGAPGPAPPASPAAPV ASDLGPRPPVSDLPRVSIYSTRRPVLARTHVQGRVNFLE RPTGWKCFVYHFAVFLVLVCLIFSVLS	
nQT1		KTVVFQEPDIGPPSEHDQLTTLHDSEGNRKMSLVGKPLTYKNYRTDQFRMRQNMKNHFLERPRGWKAATYHLAVLEFWLMCLALS VFS	
		S2	
KCNQ3		TFKEYETVSGDWL LLLLETFFAIFFGAEFALRWAAGCCCRYKGWRGLKFA RKPICMLDIFVLIASVPVAVGNQGNVLATS. LRSIRFL	232
KCNQ2		TIKEYEKSSEGALYILKEIVTVVFGVEYFVRWAAGCCCRYGWRGLKFA RKPFCVIDIENVLIASIAVLAAGSQGNVFATSALRSRFL	203
KCNQ1		TIEQYAAALATGTLFWMEILVLVFFGTEYVVRWMSAGRSKYVGLWGR LRFARKPISILDIWVVASMVLCVSGKQGVFATS AIRGIRFL	
nQT1		TMPDFEVNATIVLYLEIVFVINLATEYICRWMSAGRSRYRGISGRIFRATSAYCVIDIIVILASITVLCIGATGQVFAASAIRGLRFF	
		S3	
KCNQ3		QILRLMRDRRGTTWKL LGSALCAHSELITAWYIGFLTILSSFLVYLVEKDVPEVDAQGEEMKEEFETYADALMWGLITLATIGYGDK	322
KCNQ2		QILRLMRDRRGTTWKL LGSVVVAHSELVTA WYIGFLCLILIASFLVYLAEK. ....GENDHFDTYADALMWGLITLTITIGYGDK	283
KCNQ1		QILRLMHVDRQGGTWRLLG SVVFIHQELITTLTYIGFLGLIFSSYFVYLAEKDAVNES. ....GRVEFGSYADALMWGVVTVTTIGYGDK	
nQT1		QILRLMRI DRRAGTWKLLG SVVMAHRQELLTTVYIGFLGLIFSSFLVYLCEK. ....NTNDKYQTFADALMWGVITLSTVGYGDK	
		S4	
KCNQ3		TPKTWEGRLLAATFSLIGVSFFALPAGILGSG LALKVQEQHRQKHFEKRRKPAELIQAAWRYATNPNRIDL VATWRFYESVVSFPFF	411
KCNQ2		YPQTNWGRLLAATFTLIGVSFFALPAGILGSGFALKVQEQHRQKHFEKRRNPAAGLIQSAWRFYATNL SRTDLHSTWQYERITVTPMY	372
KCNQ1		VPQTNWGTIASCFSVFAISFFALPAGILGSGFALKVQEQHRQKHFNRIIPAAASLIQTAWRCYAA. ENPDS. STWKIYIRKAPRSHT	
nQT1		TPETWPCKIIA AF CALIGISFFALPAGILGSGFALKVQEQHRQKH LIRRRVPAAKLIQCLWRHYSAPESTSL. ATWKIHLARELPPIV	
		S5	
KCNQ3		SSQTQTYGASRLIPPLNQLELLRNL. ....KSKSGLAFRKDPPEPSPSKGSPCRGPLGCCPCGRSSQKVS LKDRVF. SSPRGVAAGKG	443
KCNQ2		.....LLSPSPKPKKSVVK. ....KKFKLDKDN. ....GVTPGEKMLTVPHI. ....TCDPPEERRLDH. FSVD. ....	457
KCNQ1		.....KLTPLGNNATGLINRLRQSTKRTPNLNNQNLANVSOATSKNLSVPRVRVHDTISLVSTSDISEIEQLGALGFSLGWKS K	
nQT1		.....RKEQLEAASSQKLG LLDVRVLSNPRG SNTK GK	

FIG. 3A

KCNQ3	.....LFTPLNVDAIEESPSKEPKVPGLNKERF.....	472
KCNQ2	GSPQAQTVRRSPSADQSLSDSPSKVPKSWSFGRSRA.....	492
KCNQ1	.GYDSSVRKS.....PTLLEVSMPEHF.....MRTNSFAEDLDLEGETLL..TPI.....	
nKQT1	SKYGGSKKATDDSVLQSRMLAPSNALHDDMRRRRRRSASLCRVVNTGHLRPLQPRSTLSDSDVIGDYSLLMAPIYQWCEQMVQRNSTPG	
KCNQ3	RTAFRMKAYAFWQSE.DAGTGDPMAEDRGYGNDFPI.....EDMIPTLKAAIRAVRILQRLYKKKFKEITLRPYDVKDVIQYSAGHLDM	557
KCNQ2	RQAFRIKGAASRQNSEESLPGEDIVDDKSCPCFVT...EDLTPGLKV/SIRAVCVMRFLVSKRKFESLRPYDVMQDVIQYSAGHLDM	578
KCNQ1	.....THISQL.....REHH.....RATIKVIRRMQVFVAKKKFQQAARKPYDVRDVIQYSQGHNL	
nKQT1	EDGVMSQLSQLSTTTCATRRTEDISDGDEEEAVGYQPQTIEEFTPALKNVCVRAIRRIQLLVARKKFKEALKPYDVKDVIQYSAGHVDL	
KCNQ3	LSRIKYLQTRIDMIFTGPPSTPKHKKSQKGSFTFPQQSPRNEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMMHQM	647
KCNQ2	LSRIKSLQSRVDQIVGRGPAITDK.....DRTKGPAAEALPEDPSMMGRGLGKVEKQVLSMEKKLDFLVNIYMQRM	648
KCNQ1	MVRIKELQRLDQSIGKPSLFSVSEKS.....KDRGSNTIGARLNRVEDKVTQLDQRLALITDMLHQLL	
nKQT1	QSRVKTIVQAKLDFICGKNIEKIEPKI.....SMFTRIAITLETTVGKMDKKLDMVEMLMGRQ	
KCNQ3	ERLQVQVTEYPTKGTSSPAEAEKKEDNRYSDLKTIICNYSETGPEPPYSFHQVTIDKVSPYGFFAHDPVNLPRGPPSSGKVQATPPSS	737
KCNQ2	GIPPTETEAYFGAKEPEPAPPYHSPEDSREHVDRHGCVKIVRSSSTGQKNFSAPPAAPPVQCPTSWOPQSHPRQCHGTSVPVDHGS	738
KCNQ1	SLHGGSTPGSGGPPREGGAHITQPCGGSGVDPELFLPSNTLPTYEQLTVPRRGPDEGS.	
nKQT1	ASQRFVSQNTSPRGEFSEPTSAQDLTRRRSMVSTDMEMYTARSHSPGYHGDARPIIAQIDADDDDEDENVFDDSTPLNNGPGTSSC.	
KCNQ3	ATTYVERPTVLPIILLDSRVSCHSQADLQGPYSDRISPRQRISITRSDTPLSLMSVNHHEELERSPSGFSISQ...DRDDYVFGPNNGS	824
KCNQ2	LVRIPPPPAHERSLSAYGGGNRASMEFLRQEDTPGCRPPEGNL...RDSDTISIPSVVDHEELERSFGFSISQSKENLDALNSCYAAVA	825
KCNQ3	SWMREKRYLAEGEFTDTPFTPSGSMPLSSSTDG.ISDSVWTTPSNKPI.	872
KCNQ2	PCAKVRPYIAEGESDTSDDLCTPCGPPPRSATGEGFPGVGVWAGPRK.	872

FIG. 3B

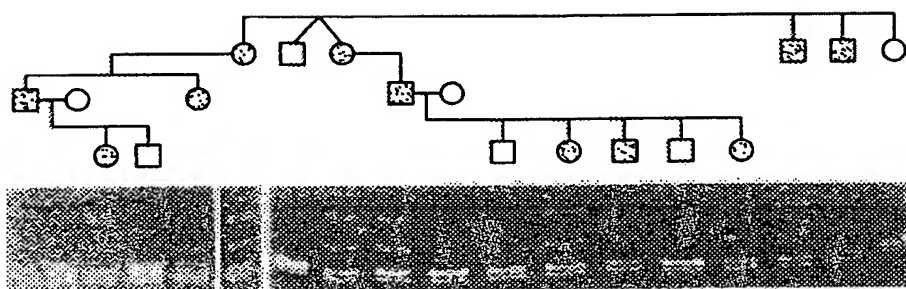


FIG. 4

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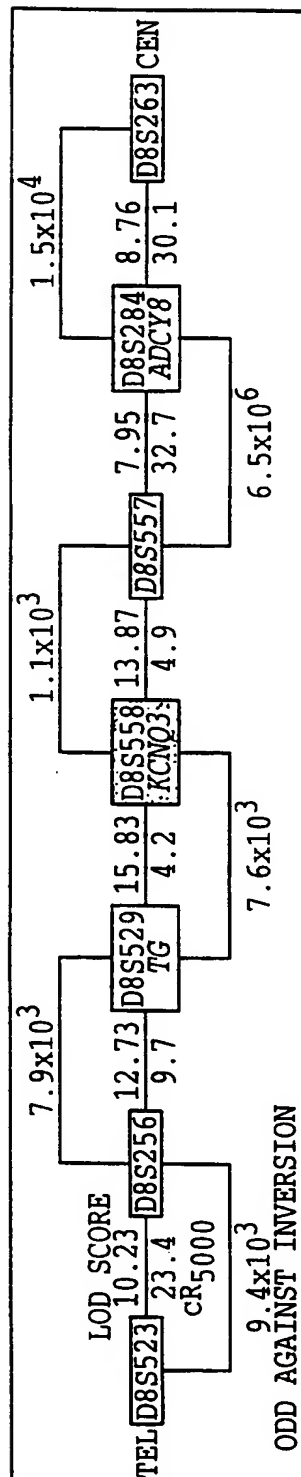
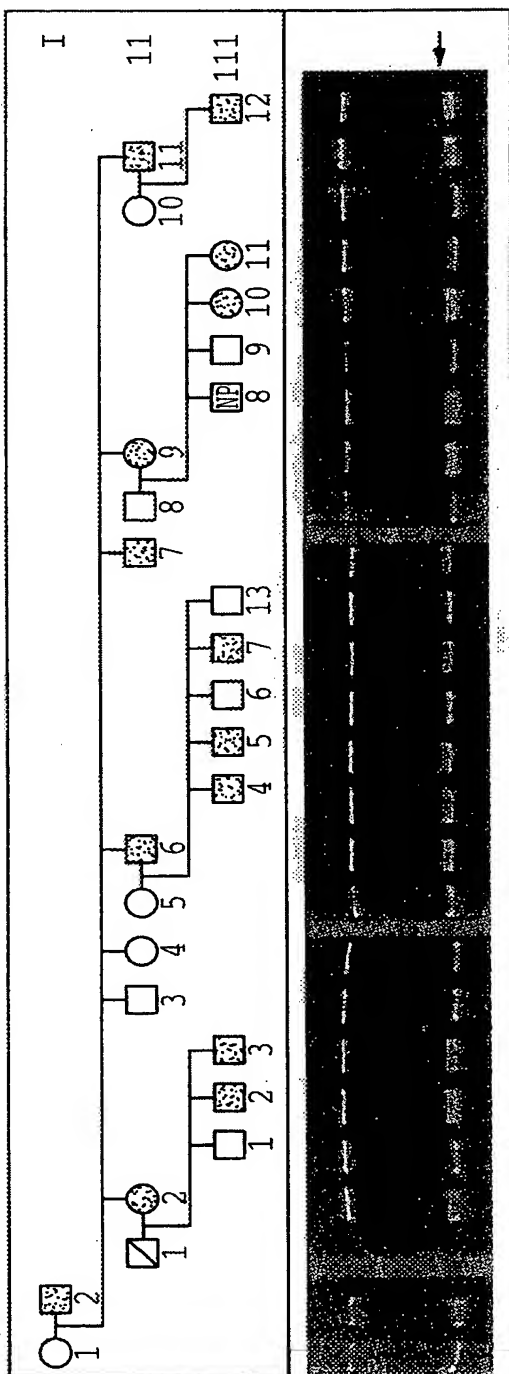


FIG. 5



**FIG. 6**

**SUBSTITUTE SHEET (RULE 26)**

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**G TTCCTCCTGGTTTTCTCCTGCCTCGTGCTGTCTGTGTTTTCCACCATCAAGGAGTATGAGA  
AGAGCTCGGAGGGGGCCCTCTACATCCTGGTGAGCCCCGAGGGAGGGCGGGGGCTGGAAGTG  
CCCAGGAAGGAGCTGGAGCTGCCTGGGCGTCTGTCTT**

**FIG. 7A**

**AGGCCTCAAGGTGGCCTCAGCTTTCCTCCCCCTGCAGGAAATCGTGACTATCGTGGTGTTTGG  
CGTGGAGTACTTCGTGCGGATCTGGGCCGAGGCTGCTGCTGCCGGTACCGTGGCTGGAGGG  
GGCGGCTCAAGTTTGCCCGGAAACCGTTCTGTGTGATTGGTGAGGCCTGGTGGGGGTGGTAT  
TGCTAGAATCAGGGCCAG**

**FIG. 7B**

**ACATCATGGTGCTCATCGCCTCCATTGCGGTGCTGGCCGCCGGCTCCCAGGGCAACGTCTTT  
GCCACATCTGCGCTCCGGAGCCTGCGCTTCCTGCAGATTCTGCGGATGATCCGCATGGACCG  
GCGGGGAGGCACCTGGAAGCTGCTGGGCTCTGTGGTCTATGCCACA**

**FIG. 7C**

**TGGTCACTGCCTGGTACATCGGCTTCCTTTGTCTCATCCTGGCCTCGTTCCTGGTGTA CTTG  
GCAGAGAAGGGGGAGAACGACCACTTTGACACCTACGCGGATGCACTCTGGTGGGGCCTGGT  
GAGTTGTGGTCATTGTGGTTTTCCCTTTCCCTGCTGATACACCCCTGTCCCTGTGCTGGGAC  
CAGGCTCTCACTGGCTGAGCCTGCTCCAT**

**FIG. 7D**

**GCAGGCCCTTCGTGTGACTAGAGCCTGCGGTCCACAGATCACGCTGACCACCATTTGGCTAC  
GGGGACAAGTACCCCCAGACCTGGAACGGCAGGCTCCTTGCGGCAACCTTCACCCCTCATCGG  
TGTCTCCTTCTTCGCGCTGCCTGCAGTAAGTCCAGCTGCCCTGCCTTGGAGGGGGAC  
GAGGTCTTGTAGGCTCCCGAGGTGACCACAGGCCCTGGGCACAGTTCCCTAGGT**

**FIG. 7E**

**ATGGTCTGACCCTGATGAATTGGGGTGTGGGGGGTCCCTGGGGTGTGACCTGACCCTGATGA  
ATTGCAGGGCATCTTGGGGTCTGGGTTTGCCCTGAAGGTTCAAGGAGCAGCACAGGCAGAAGC  
ACTTTGAGAAGAGGCGGAACCCGGCAGCAGGCCTGATCCAGGTGAGTCCAGGTGTCCCCCG  
GGACCAGCACAGCCCTTGTCTTGGTCCCACCTTGTGAGGAGTGGAGGGCCG**

**FIG. 7F**



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AGCTGTGCAAGCAGAGGGAGGTGTCCCAGGACTCGGGAGGGTGAGACGCTCACTCCCCTCTC  
CTTCTCTTGCCCCAGACTTATCCCCCGCTGAACCAGCTGGAGCTGCTGAGGAACCTCAAGA  
GTAAATCTGGACTCGCTTTCAGGTCAGCTGGGGAGCTCCAGGTGGGGCGGGTGGGCGTCTCA  
GTCTCCTGGGGGCCCCAGCTGCCACAGAAGACACGCCAGGACAG

FIG. 7G

CCCAGGACTAACTGTGCTCTCCTCATTTCCAGTAAAGGCAGCCCGTGCAGAGGGCCCCTGTG  
TGGATGCTGCCCCGGACGCTCTAGGTACNRCGGAACACRMSSCACGGACTGACGGCTGCTGC  
ACGG

FIG. 7H

GCAGAGTGACTTCTCTCCCTGTTTTTCTGTCTGTCTGTCTGTCTGTCTGCGTTCCCGTGGGAGC  
AGCCAGAAGGTCAGTTTGAAAGATCGTGTCTTCTCCAGCCCCGAGGCGTGGCTGCCAAGGG  
GAAGGGGTCCCCGCAGGCCCCAGACTGTGAGGCGGTCAACCAGCGCCGACCAGAGCCTCGAGG  
ACAGCCCCAGCAAGGTGCCAAGAGCTGGAGCTTCGGGGACCGCAGCCGGGCACGCCAGGCT  
TTCCGCATCAAGGGTGCCGCGTCACGGCAGAACTCAGAAGGGGTGTGGCCGCATCCTCTCCT  
GGTCCATC

FIG. 7I

CCCTCACGGCATGTGTCCCTTCCCCCAGAAGCAAGCCTCCCCGGAGAGGACATTGTGGATGA  
CAAGAGCTGCCCCCTGCGAGTTTGTGACCGAGGACCTGACCCCGGGCCTCAAAGTCAGCATCA  
GAGCCGTGTGGTGAGGCCCTGCCAGCCGGAGCCTGGGGGAGTGAGGAGGGGCCTCCCGC  
T

FIG. 7J

GGTCTCTGGCCCAGGGCTCACAGCCCCACCCACCCCCCTGCAGTGTGATGCGGTTCTGGTG  
TCCAAGCGGAAGTTCAAGGAGAGCCTGCGGCCCTACGACGTGATGGACGTGATCGAGCAGTA  
CTCAGCCGGCCACCTGGACATGCTGTCCCGAATTAAGAGCCTGCAGTCCAGGCAAGAGCCCC  
GCCTGCCTGTCCAGCAGGGGACAAG

FIG. 7K

CCCAGCCCAGCAGCCCCCTTTTGCAGGTCTTGTCCATGGAGAAGAAGCTGGACTTCCTGGTGA  
ATATCTACATGCAGCGGATGGGCATCCCCCGACAGAGACCGAGGCCTACTTTGGGGCCAAA  
GAGCCGGAGCCGGCGCCGCGGTACCACAGCCCGGAAGACAGCCGGGAGCATGTCGACAGGCA  
CGGCTGCATTGTCAAGATCGTGCGCTCCAGCAGCTCCACGGGCCAGAAGAACTTCTCGGCGC  
CCCCGGCCGCGCCCCCTGTCCAGTGTCCGCCCCTCCACCT

FIG. 7L

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**CTCCACGGGCCAGAAGAACTTCTCGGCGCCCCGGCCGCGCCCCCTGTCCAGTGTCCGCCCT  
CCACCTCCTGGCAGCCACAGAGCCACCCGCGCCAGGGCCACGGCACCTCCCCGTGGGGGAC  
CACGGCTCCCTGGTGCGCATCCCGCCGCGCCTGCCCACGAGCGGTGCTGTCCGCCTACGG  
CGGGGGCAACCGCGCCAGCATGGAGTTCCTGCGGCAGGAGGACACCCCGGGCTGCAGGCCCC  
CCGAGGGGAACCTGCGGGACAGCGACACGTCCATCTCCATC**

**FIG. 7M**

**TGGAGTTCCTGCGGCAGGAGGACACCCCGAGCTGCAGGCCCCCGAGGGGACCCTGCGGGAC  
AGCGACACGTCCATCTCCATCCCGTCCGTGGACCACGAGGAGCTGGAGCGTTCTTCAGCGG  
CTTCAGCATCTCCAGTCCAAGGAGAACCTGGATGCTCTCAACAGCTGCTACGCGGCCGTGG  
CGCCTTGTGCCAAAGTCAGGCCCTACATTGCGGAGGGAGAGTCAGACACC**

**FIG. 7N**

**GTGGCGCCTTGTGCCAAAGTCAGGCCCTACATTGCGGAGGGAGAGTCAGACACCGACTCCGA  
CCTCTGTACCCCGTGCGGGCCCCCGCCACGCTCGGCCACCGGCGAGGGTCCCTTTGGTGACG  
TGGGCTGGGCCGGGCCCCAGGAAGTGAGGCGGCGCTGGGCCAGTGGACCCGCCCCGCGGCCCTC  
CTCAGCACGGTGCCCTCCGAGGTTTTGAGGCGGGAACCCCTCTGGGGCCCTTTTCTTACAGTAA  
CTGAGTGTGGCGGGAAGGGTGGGCCCTGGAGGGGCCCATGTGGGCTGAAGGATGGGGGCTCC  
TGGCAGTGACCTTTTACAAAAGTTATTTTCCAACAGGGGCTGGAGGGCTGGGCAGGGCCTGT  
GGCTCCAGGAGCAGCGTGCAGGAGCAAGGCTGCCCTGTCCACTCTGCTCAAGGCCGCGGCCG  
ACATCAGCCCGGTGTGAAGAGGGGCGGAGTGATGACGGGTGTTGCAACCTGGCAACAAGCNG  
GGGGTTGNCCAGCCGANCCAAGGGAAGCACANAAGGAAGCTGTNCCCTAAGACCTNCCNAA  
AGGCGGCCCTGTTTGGTAAGACTGCGCCTTGGTCCGGTGGGTTCGGGCAGCAAAAGCGGGTTT  
TGCCGCCCTGTTCGTG**

**FIG. 7O**

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GGCGACGTGGAGCAAGTCACCTTGGCGCTCGGGGCCGGAGCCGACAAAGACGGGACCCTGCT  
GCTGGAGGGCGGGCGCGACGAGGGGCAGCGGAGGACCCCGCAGGGCATCGGGCTCCTGG  
CCAAGACCCCGCTGAGCCGCCAGTCAAGAGAAACAACGCCAAGTACCGGCGCATCCAAACT  
TTGATCTACGACGCCCTGGAGAGACCGCGGGGCTGGGCGCTGCTTTACCACGCGTTGGT

**FIG. 8A**

aacttctctcacattgttttatttaactgggatgattgtttccgcctgccttgcaggtttgt  
cgtgaagattgaatgggatagcatataaagcacatgtcaatgtccagcagaagttgcagctt  
catcctggaagacacctttcccatcttagcctcaaagcaagccatgactcaaagggttcctt  
agtccatttctttcttccctctagGTTCTTGATTGTCCTGGGGTGCTTGATTCTGGCTGTC  
CTGACCACATTCAAGGAGTATGAGACTGTCTCGGGAGACTGGCTTCTGTTACTGgtaagatt  
gcattctggggtaaatgcttctggttgggcttccagagtgatgaaaaggaggttgccttgg  
gtgcactcctccctgactggttgcagcttcttgtagtctccagtcaagtccaggcccaagga  
aaagcaaagcctccattactyggatggcccgccatgggcacatgtgggtgagaatggca  
ttcctggtaaagctttgctattccacattagagagaaggggaaataaagtcaaagcaaaaac  
caatgcatgttattaaattataaaatacagcttcccaatcctctgaaaggtaacacaaaggc  
atgtttcattctaaaacctgtctctgctttttcttccctgggatcctacaatctaaactcaa  
ggatctctcattctctccaaggccaggtacagaattccatttatacacgaaacctctaaatc  
tccctcctggggcctgcatttgttttctctctgtcctccatcaggtggtgtgatggaaa  
cagagcaggattagaattcctgggcaagtcagcta

**FIG. 8B**

agggtgcctggcccaggagccaggctttataaccattagccacaattagcaatgccagggt  
acaggcactgggtggaatttacagattgtgtttcactcatatctctcttttcaacccaactg  
cattcttgggggcttttcatcattaaagggaacttttaagctgacctattggaacaaaaa  
catagaaaaaagaacgagtaatcactgtgccagggttaacagcattaaggacaattagcaca  
tcagaatgaagatgggaggcctccaaactgaatggcggtgatggacctgttctcccggttcc  
ccttccccacccccatccccagccatccctgcccaaccagacaaccagcaacagcacaataatg  
gagttcttcagaacttccgaatagaaatcaccagctcccagacaagtggatctcgggttaatca  
gtgcctctccatattgctcttccatgcagGAGACATTTGCTATTTTTCATCTTTGGAGCCGAGT  
TTGCTTTGAGGATCTGGGCTGCTGGATGTTGCTGCCGATACAAAGGCTGGCGGGGCCGACTG  
AAGTTTGCCAGGAAGCCCCCTGTGCATGTTGGgtaagtcctgacctgagcctccagcctcc  
tcagttcccttcttttggggcattgttttctctgagaaaagtttaagcagctattctgggaaa  
tcacgcggcactgtggaggccagctcagccctgacgctgcctcgatgagaaggacatgtc  
aaccttctgggtcctcaaattcctcctctgtgactggtccttataaggactgcacaggaca  
gggattcttatttggcagggtagggtgtcactcttggcaattgggttgtggag

**FIG. 8C**

gggggccttggtaaattgctgctctggagccagcattaaagtgtggtaggcctatgctactt  
ctggccagggtggccttggaaagtcactcaggctcagagcttcagtttctcatcagtcattg  
gagaataatcccacttaccatgtgttgttgggtggcaagattcaacagcagtgctcagattgt  
cccactgcttggcacatgctgatctgccagcaaacagcacctatgatgacgccattgcttcc  
gcatgaccttcccttccctcttccctccactctgtctgtcctctctcccagACATCTTTGT  
GCTGATTGCCCTCTGTGCCAGTGGTTGCTGTGGGAAACCAAGGCAATGTTCTGGCCACCTCCC  
TGCGAAGCCTGCGCTTCCTGCAGATCCTGCGCATGCTGCGGATGGACCGGAGAGGTGGCACC  
TGGAAGCTTCTGGGCTCAGCCATCTGTGCCACAGCAAAGtaagtgtggtggagaaactgca  
ggaccacatgggcttcccacccacctatgcccttccatgacatcccttcccttgcagtgctcc  
ccagaaggcagtcattctgccacccttgatgataacgacaaaagagggaaggaggaggagaa  
acaggaagtgcgggggctggggtagggc

**FIG. 8D**

gaaaatcaaaacagatcccaattctggaagtctccggctatagtcaaagtatcacgtgacag  
ttcaagcagcgtaaaaatatttttaaactcagttaacattactgggcattctattttgtgcagt  
accccttactggcagttttataaagggttatctcacttttttctaatacatgcattaggtattat  
tatcccacatccctatagaaaaaaccaatatgcaacaggggctaaggggccttgcccaggccct  
cacacctggaaagtggcagtggtcagaattggaaccaggtcttcctgacttcaagggtcatt  
tcaacttaaccaagctccctactctcttcaagagaaggaagggtctcttcccccttcccttct  
tagtacagtggtgtcactgcaaggacttgaagtgaattgagccctacagtccccattacc  
tggaatggagcgggaatgctgggacagtcctagctgggggctgactgcctgcctgcctctcc  
ctcagGGCATCCTGGGGTCCGGGCTGGCCCTCAAGGTGCAGGAGCAACACCGTCAGAAGCAC  
TTTGAGAAAAGGAGGAAGCCAGCTGCTGAGCTCATTCAGgtctgtctgcctgggaatgaact  
ggaatgggattaagatccatgcatatgtacatacgtgtgtgtgtgtgtatgtgtgcatgtgt  
gcacatgtggaggggacatactcatgaactgggacaggaccgattccatgtgtgtctgtgtg  
tcttgtgtgtctgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtatgaatgtgccc  
aggcaggagcaggcctgcttgacatgcttacttgtggatggctatggggagtttccatggg  
tatctatttcacctgttcttctgtgtactgaagggtgacaatcctgtcactctctcattcagt  
tcttaagccaagaaagaaatagacacagaactcaaggaccaacctatcatcttttttttgat  
acgggtgggttttttgaggttttttttgagactctcttgtccaggctggattgtagggtgcat  
catactcactgcagcctccatcncagg

**FIG. 8G**

aactcttggcctcaaagtgatcctcccaccttgggtctcccaaagtgctgggattacagggcgt  
gagccatagcaccggcctttagtacttggtcctttcagggattttatgcctactactctctt  
ctctccctccactccagttcatctctccattccccactcaccacaacaccaattatagctc  
caagatgggtcaaggaagtttttcttcccaaagcagcttcaaaaagccaagaatctcggtttt  
tctgaatggtgggtcaatgcacattcaaattcttaggagtcagggcttaaacattgttttg  
ttgggtgtgggagtctgtgcgaaagtttcgggtggtgccactcattgttgccctcttttctg  
cccctcagGCTGCCTGGAGGTATTATGCTACCAACCCCAACAGGATTGACCTGGTGGCGACA  
TGGGATTTTATGAATCAGTCGTCTCTTTTCCTTTCTTCAGgcaagtggggactcacctgaat  
gctcagggcggtgaccagccatctctcctgcgggtctgtattcgtgtctggccctcacgggtccc  
tggaacactcttcagggcaatgttccccaatttgggctgcaccctagaattatctggtag  
cttaaacagttctggctggggcgcggtgggtcacaccataatcccagcactttgggaggcgcg  
aggcgggtggatcacctgaggtcaggagttccataaccagcctggccaacatggtgaaatccc  
gttctactaaaaatgcaaaaattaccggggcggtgggtgtgtgctgtaatcccagctac  
tcaggaggctgaa

FIG. 8H

gactgaatggacttagtacaagttgggtcataaggggtcccgagggggtacaggaagatgctgg  
ggtaggagtgatggcagattatacgttcttatatacaagcagggatgaggaagctgttaaa  
aatcagacattgctttttataaacagagcatgtgcattgttttattcctggtagggagagt  
gaattatgtctggcttttcattttctatagctgcaccgttcaatatggtagccactagcctc  
atgtggctagtggagaatttgactgttagcactgcaattgaggaacagatttttttaattttaa  
agtaaataaccttctatgactaatgactactctattggacagcacagctctggaattgtag  
ctatgagaactgaaatggagataagaagacttcgccacgatgtagaaaatacttgaccaag  
aacaggtagttcattgtgtgaaccaggacttggttccttttaacagGGTCAAGATTATTGGAGT  
GCTTAGAAATGGAGAAAGGGGACTATATGCACTAGTCATTTCCTATGGCCAAATAACATTGG  
ATCTGCTTTCATACATACTATCTTATTTAAGCTTTAGGATGTCCTGGAAGgtaagtagaagg  
ggtaactccatttttcataacccatttttataggtaaagaataagagagtcaatgagatta  
attagcttgcttaatatcgctcagctgataagtgatggaacaaagattggaactcaggtctt  
gtgccaaaacctatgtttttattttgcatgtatctctgggaagaaaacattatttagggag  
aaaactggataaaaagtaagatgacacaaggggttggttgataataagaccattttttgaaga  
ttgttggttggtggtcaaactgagtaaaatgtgtgagagtggttg

**FIG 8I**

FIG. 81

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acgcaagtcctggaatagacccaaagtttcttgagtcctgagccttgtattagaagaaggag  
ccacttcctcctgccttcttgcccttctctgaagcctccttgagctgtgatattgaagtggcc  
ctaagctagaaatcttctctcctccttgaggccatacacttttctggtaaattaatgaatg  
aaataactaccatgttaatgatcccattttacagtgatggaagatgaagatcagagaagggtg  
agtgatttgaccacagtcacagagctggtaaacttggactctaactctgggtgtgtctggctc  
cagcatccactcaacgactccccagtgaccacttttcatgtccactgttcattctttcagGA  
ACTCATCACGGCCTGOTACATCGGTTTCCTGACACTCATCCTTCTTCATTTCTTGTCTACC  
TGGTTGAGAAAGACGTCCTCCAGAGGTGGATGCACAAGGAGAGGAGATGAAAGAGGAGTTTGAG  
ACCTATGCAGATGCCCTGTGGTGGGGCCTGgtgagtcactaccttggaggccaattctgtga  
gattgatggtcaagagtcagagagaggtggagggcatcacatgagcatgttcagccaggcag  
ctgcattctgcagtcagaggtaagctctagaccaatttcagctcagaacctgctgacagaag  
accctccttcaaggtgggcacttgggaattgacttttctctagcgtttataagaagccagggc  
ttggaacagcctggttgcatggtcggttatggacttagccttattagtcataggctattttc  
agccaagccatgcatgtgcaaacaacccagtgacagatacacatgtgtgtgtcacacagacc  
tgtgtgtgcacaaccctacaccacacaggacacacagtactaaagctggcattcactgaagg  
ctttctttgctccagagcatctctctgggtgctttactttcact

## FIG. 8E

gaacagatacatgcacagacattagacatacacacatatatacacaaatacacatacaaatatac  
tcacaagcacacatatattcacaacacatggctataaataaaatcacaaattcacaaatatac  
acacacatgaatgctcgtgtacatacacatttgcaattgctgaaatatttggtgactgacta  
aggtaggaaacccttaacttatcaacaagtctcaaggcatccatataagttagtaggtactt  
ggtgtcttttctcctaagggaaccttggttatgaatgggagcattgcccagctgatggagag  
gcttacaggtagagctcagttaacacgtttctgatattcctctccatgtgggtactccatgtc  
tgaactcttctctcttcagATCACACTGGCCACCATTGGCTATGGAGACAAGACACCCAAAA  
CGTGGAAGGCCGTCTGATTGCCGCCACCTTTTCCTTAATTGGCGTCTCCTTTTTTGCCCTT  
CCAGCGgtaagtacctttgatataatgacatccccaatgtgacgtgcaggacccttaccgcc  
tggtgccagctcaactttccagtggtcatcttctatcctcttataccctaccaactccctagc  
cattcccttaagcatgatgatcctgcctttttgccacaggeccctgctgctttcctctgccaa  
agatttcttcacacatcaactcctctttcaatgctgccttcttttaggctgagctagtgcgtc  
tgggcataactctgggaataattctgtaaaggagtttctggccctatgctaggattacacat  
ttctagatctgccttccccagaggactgtgaattccttgggttctgggattatatttttcat  
tcatgcatttccagtgcttgcacggagcaggtccttcatttatgtagtcccttctcttg  
tcctgttacntactggcttatgtaaaaaatacatgtctctcaagaataagtctgacctatga  
tagagtaactnccccaacgccagtggtccaggtacgtaataataatgaaagcagattgcatt  
tggttgaactcactgtggcctgaatcatgccaaaagggttaccacatcatctcatttaate  
t

## FIG. 8F

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tacaatgtgatccacgtaataatgacagagtaccattccacttgtgaggggatttgcctcagt  
gtagaccttgggcaattgaataagaacccctaggagggccctggaggtgtacataaaggat  
gagtaggcctgttccaagcagggaaaagaggaagggagttccaggcagaaggagagcctgag  
aaaaggccttggagtcataatgtgttaaagcacgggctgggtgcacctcccagttaggagt  
agggtccagagccagatcacctgggtttggatcctgactttgctgcctcctaactgtgagc  
ccttgagcaattcatttaacccctctgtgcctcaattttctcctcagggaaatgggatgata  
atagtacttcataggggtgttatgaggattaattgagtttaagacaatgttcgctatgatgac  
aatggtagtgacaaagtattgggggtgtgtgactgctacattatgacattcctggtttcctg  
gtctgtctccaccaccaataaatttccctgagctcaacatgagagctggggcagagtaagt  
ctcagcaaccatttttctggatgaataaatgaatgaatgagtggtgaaaagagccctgaaaa  
cctcagagccaacgggagtagcatgggctggggtctggatgggttaaaccgcctccttcatt  
gggtccctccacactgaccatcctgtcctagagctcaactctgctccatcatcttcagagag  
aagctttgcagcaatctttcgaggaaggatacagctgtttcacgtaatttatgctttattct  
ttctccctcttctctttctagGAAAGAACAGCTGGAGGCAGCATCCAGgtaagtttctgatt  
atgaattcccttcttcacatctctgtgtcaagacagagcatcctgctccatatgggtgtaggg  
ccccatgggaggtcatgctgggtcccaagatagagttcttggggtcacactgttgctgaccac  
catagtccctctgcctgggttcttctggttgatctgagggaaacttaataggaatcatggca  
gcagcctcttattgaggggtctgggttctgtgtcaggagttctgcatatgttatctcatttgg  
tcttcacaaccacaatgtaacgataggccctaatatcatcccttgtggatgaggagattgtg  
gctcagagaggttgggttgagattgagtggaacaaccaaattcatagccagg

FIG. 8J

aagaagtgttgctttacgtccatttgtgtggccagtttcttttcaaggaggaatcctttgat  
aaggatttgcctgtctaaatcactatctgggtaccatgggatgatacacaggaaaggcagga  
agttattgatgcaggaaatgggcatgggaaagatgaatctctgcagcatactaggatgagct  
aggcaatttatagcgggcacctcatgtaagctacatttaatcgatgggaaaattgacattc  
agagaagtcttgcccagggtataagagctagcaggctgtggagctaggatttgaaccacgcc  
ctgtccgattccaagctgctgagtcagattcagcactgtgaaatgcacgggtccccatttctc  
cttgaggagaaatgtgtgagtccttatggagggatgggaaattttaagagcctgcactgaag  
gaggaaaattgttcacttttgcttattttgagCCAAAAGCTGGGTCTCTTGGATCGGGTTCCG  
CCTTAATCCTCGTGGTAGCAATACTAAAGGAAAGCTATTTACCCCTCTGAATGTAGATGCCA  
TAGAAGAAAGTCCTTCTAAAGAACCAGCCTGTTGGCTTAAACAATAAAGAGCGTTTCCGC  
ACGGCCTTCCGCATGAAAGCCTACGCTTTCTGGCAGAGTTCTGAAGgtaatgcctttttatc  
tccctccctgtctcttccacttcttccctccccaagtccacttccctcctcacctctccctt  
tgcccacttaagaacctttgactccacaaggtaactctctcccttccctcgacaagccaact  
tcttgcttccctaactcctcctgtcccttgggctgaggcattgtgatgtattcccaggagtc  
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ccccgaccccccgccccatgattggattgttatggaggtcaacttgaaggatggggcggtgc  
caggtgcaaagcaatttagagaccaggggcaggggaagagtggcagaaaagcgccctctggag  
gctgtaggagtcattggcctcatgtgcctcttttacttatgcaaaggaggacatgcagaaaa  
gcctgtttcctcagtgcttgagcccaccagggccctcaatcctcattgtatcattca

FIG. 8K

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ktttctctcmaaggcctctngatgtgtgsggctcagaaagtgacktctccaagggtcaccagga  
tagagacttgasagagcaaawakcccagctgaggsctgcacagtgkgtgktgkttgctggst  
tcwgtgtcsttttgstggctkytggctctgggggccaytctggaactgsggagctcacttctc  
ctccctgctagccttttccctcactaccagtcagtgagtgcgacacttttgacttggacttc  
tgggtaatagaatgaggggtgccaagaaaggctgaacagcatcacagcttgagaataccgtgg  
agtcttgcaacgtggaaataaagactctggggattgacacatccagaggcgtggaaggcttt  
gaccgaacagtgggggtccccaagccttttccaggtctgtggcctgccgttcataatgtgtgtc  
tccctcccagATGCCGGGACAGGTGACCCCATGGCGGAAGACAGGGGCTATGGGAATGACTT  
CCCCATCGAAGACATGATCCCCACCCTGAAGGCCGCCATCCGAGCCgtcaggtaatgcccc  
acgggtcccacctgtgcctgtgtgcctcccccgctccagctcaactcccacaggaaggggctt  
ataaaaattatcttgcacttttggaaggggggaagagaagccccctccactaaccctgagttagg  
tccttgaagtatgtaaatactgtatgctgccccagaaaaaatgatccagacgttagcaagtc  
atgatgggtgactcgtaggtgcctgccttgttataaacacgccccacagccctcctgacagt  
atttccacctgctatgttctgctctgtctgtaactaccatgtatttttaagggtgtcagagt  
ggagggttttcttccctgtagaggcttcttgcctcaaaatggtttttcttctgcctaacttcat  
ccatatagtttgttttaattagttcgcattttttaacaagataataaattatagttttttt  
gtctgtatcagcagagaccataatccattctaccta

## FIG. 8L

agcagtgtgacagtgattaagagcaccagccttgtcagcaccctgtctgggtttgaggacca  
gctcagcccttattagctatatggccctgggatgatgctgaagggttcaaatccacaatcaca  
tcatctataaatggatctgttatccaggattgttcataaagcattaattaagctcatggctc  
ggcatacagtgaatgctcaataaatgttagctcttattaataactatgatttacttattatc  
aatgattgaaggggagtaatcctgatggagatgtactaactctgtgtgttccaaggggtaga  
accagaaccaaacggttggaagttcttccagcaagctcttttatctttgggttcttttctcccc  
ctgccctggagtttgctagccttctgttatagctccccgcactctccacatgggatgcacaa  
atgcctctactttgccttgagAATTCTACAATTCCGTCTCTATAAAAAAATTCAGGAG  
ACTTTGAGGCCTTACGATGTGAAGGATGTGATTGAGCAGTATTCTGCCGGGCATCTCGACAT  
GCTTTCAGGATAAAGTACCTTCAGACGAGgtgagacagtcacatctggaggggactgcgctc  
ccctcaaagccctatgaaccttagagtttaagggtgagaggtattcagaaataattcaaaatg  
cagggagagatttttaagaagacaaatatccacgaagccttgtggatgtctaggccaacaaag  
caccagatcggacagactgtgaaatagctgtatgacattgccatggccaagggtcagcacct  
gatcaggcctgtcagagaggagaaagcacacatttaaatggcttctgactgtgatgctttcg  
atgttgccaacaaaacaggatcatccgaattaaaccgaatccagctgcctaattaattctca  
atacaattctttaccatattttaaaaatgttcatcaggtattacttataatagttgaaagata  
tggaatagcatcaatgcctaataa

## FIG. 8M

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[illegible]

FIG. 8N

cgggtgacctgtaatcccagctacttggggagggctgaggcatagcactgcntgaacccgggagggcggaagt  
agcaatgagcccagatcgcgccactgcactccagcctgggtgacagaactgagcttcgtctcaaaaaa  
aaaaaaaaaaaaaaaaagaatatttctcccaaccaatagcaacgatccccaccctcagagaaagt  
gtaattcacagctcctttgattttccagGTTACGGACATCCGGAAGAAGCTGGACTTCCTCGTGGATAT  
GCACATGCAACACATGGAACGGTTGCAGGTGCAGGTACGGAGTATTACCCAACCAAGGGCACCTCCTC  
GCCAGCTGAAGCAGAGAAGAAGGAGGACAACAGGTATTCGGATTGAAAACCATCATCTGCAACTATTC  
TGAGACAGGCCCCCGGAACCACCCTACAGCTTCCACCAGGTGACCATTGACAAAGTCAGCCCCTATGG  
GTTTTTTTGCACATGACCCTGTGAACCTGCCCCGAGGGGGACCCAGTTCTGGAAAGGTTTCAGGCAACTCC  
TCCTTCCTCAGCAACAACGTATGTGGAGAGGCCACGGTCTCTGCCTATCTTGACTCTTCTCGACTCCCG  
AGTGAGCTGCCACTCCCAGGCTGACCTGCAGGGCCCCCTACTCGGACCGAATCTCCCCCGGCAGAGACG  
TAGCATCACGCGAGACAGTGACACACCTCTGTCCCTGATGTGGTCAACCACGAGGAGCTGGAGAGGTC  
TCAAGTGCGCTTCAGCATCTCCCAGGACAGAGATGATTATGTGTTGCGCCCCAATGGGGGTCGAGCTGG  
ATGAGGGAGAAGCGGTACCTCGCCGAGGGTGAGACGGACACAGACACGGACCCCTTCACGCCCAGCGGC  
TCCATGCCTCTGTCTCCACAGGGGATGGGATTCTGATTACAGTATGGACCCCTTCCAATAAGCCCATT  
TAAaagaggtcactggctgacccctcctttgtaatgtagacagactttgtatagttcacttactcttaca  
cccgacgcttAccagcggggacaccaatggctgcatcaaatgcatgcggtgtgcggtggggcccccacca  
ggcaggggcttcccacaGcctcttcttccccatgtcaccacaacaaagtgttcttttcagcatggnt  
tgcattgactttacactatataaatGtttccgctaattctcttctaggatacacatttatctgtgttctt  
acttttattcacgattggaccagtacagggGaaattactgatgagccatgctatttgtctgtttgggt  
ggctgggtatgggttttgggtttggttaagcaa

FIG. 80



## SEQUENCE LISTING

<110> Leppert, Mark F.  
Singh, Nanda  
Charlier, Carole

<120> KCNQ2 AND KCNQ3 - POTASSIUM CHANNEL GENES WHICH ARE  
MUTATED IN BENIGN FAMILIAL NEONATAL CONVULSIONS (BFNC)  
AND OTHER EPILEPSIES

<130> 2323-134

<140> U.S.  
<141> 1998-10-23

<150> 60/063,147  
<151> 1997-10-24

<160> 129

<170> PatentIn Ver. 2.0

<210> 1  
<211> 3232  
<212> DNA  
<213> Homo sapiens

<220>  
<221> CDS  
<222> (128)..(2743)

<220>  
<221> mutation  
<222> (975)..(976)  
<223> There is an insertion of a GT between nucleotides  
975 and 976 in kindred K1504.

<220>  
<221> mutation  
<222> (978)  
<223> The mutation A to G occurs at this base in kindred  
K3904.

<220>  
<221> mutation  
<222> (1043)  
<223> The mutation G to A occurs at this base in kindred  
K1705.

<220>  
<221> mutation  
<222> (1691)..(1703)  
<223> The thirteen nucleotides from 1691-1703 are  
deleted in kindred K3369.

<220>  
 <221> allele  
 <222> (1039)  
 <223> This polymorphism of C to T was seen in 7.0% of the control population.

<220>  
 <221> allele  
 <222> (1846)  
 <223> This polymorphism of C to T was seen in 0.57% of the control population.

<220>  
 <221> mutation  
 <222> (1469)  
 <223> The mutation C to T occurs at this base in kindred K1525.

<220>  
 <221> mutation  
 <222> (1094)  
 <223> The mutation C to T occurs at this base in kindred K4443.

<220>  
 <221> mutation  
 <222> (1125)  
 <223> The mutation G to A occurs at this base in kindred K4516.

<220>  
 <221> mutation  
 <222> (2736)..(2737)  
 <223> There is an insertion of GGGCC between these two nucleotides in K3963.

<400> 1  
 gagtgcggaa ccgcgcctc ggccatgcgg ctcccggcgg gggggcctgg gctggggccc 60  
 gcgcgcggccc ccgcgctccg ccccgctga gctgagccc gaccgggggc gcctcccgcc 120  
 aggcacc atg gtg cag aag tcg cgc aac ggc ggc gta tac ccc ggc ccg 169  
           Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Pro  
           1                          5                          10  
 agc ggg gag aag aag ctg aag gtg ggc ttc gtg ggg ctg gac ccc ggc 217  
 Ser Gly Glu Lys Lys Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly  
   15                          20                          25                          30  
 gcg ccc gac tcc acc cgg gac ggg gcg ctg ctg atc gcc ggc tcc gag 265  
 Ala Pro Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu  
                           35                          40                          45

gcc ccc aag cgc ggc agc atc ctc agc aaa cct cgc gcg ggc ggc gcg 313  
 Ala Pro Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala  
                     50                                    55                                    60

ggc gcc ggg aag ccc ccc aag cgc aac gcc ttc tac cgc aag ctg cag 361  
 Gly Ala Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln  
                     65                                    70                                    75

aat ttc ctc tac aac gtg ctg gag cgg ccg cgc ggc tgg gcg ttc atc 409  
 Asn Phe Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile  
                     80                                    85                                    90

tac cac gcc tac gtg ttc ctc ctg gtt ttc tcc tgc ctc gtg ctg tct 457  
 Tyr His Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser  
                     95                                    100                                    105                                    110

gtg ttt tcc acc atc aag gag tat gag aag agc tcg gag ggg gcc ctc 505  
 Val Phe Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu  
                     115                                    120                                    125

tac atc ctg gaa atc gtg act atc gtg gtg ttt ggc gtg gag tac ttc 553  
 Tyr Ile Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe  
                     130                                    135                                    140

gtg cgg atc tgg gcc gca ggc tgc tgc tgc cgg tac cgt ggc tgg agg 601  
 Val Arg Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg  
                     145                                    150                                    155

ggg cgg ctc aag ttt gcc cgg aaa ccg ttc tgt gtg att gac atc atg 649  
 Gly Arg Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met  
                     160                                    165                                    170

gtg ctc atc gcc tcc att gcg gtg ctg gcc gcc ggc tcc cag ggc aac 697  
 Val Leu Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn  
                     175                                    180                                    185                                    190

gtc ttt gcc aca tct gcg ctc cgg agc ctg cgc ttc ctg cag att ctg 745  
 Val Phe Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu  
                     195                                    200                                    205

cgg atg atc cgc atg gac cgg cgg gga ggc acc tgg aag ctg ctg ggc 793  
 Arg Met Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly  
                     210                                    215                                    220

tct gtg gtc tat gcc cac agc aag gag ctg gtc act gcc tgg tac atc 841  
 Ser Val Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile  
                     225                                    230                                    235

ggc ttc ctt tgt ctc atc ctg gcc tcg ttc ctg gtg tac ttg gca gag 889  
 Gly Phe Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu  
                     240                                    245                                    250

aag ggg gag aac gac cac ttt gac acc tac gcg gat gca ctc tgg tgg 937  
 Lys Gly Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp  
                     255                                    260                                    265                                    270

ggc ctg atc acg ctg acc acc att ggc tac ggg gac aag tac ccc cag	985
Gly Leu Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln	
275 280 285	
acc tgg aac ggc agg ctc ctt gcg gca acc ttc acc ctc atc ggt gtc	1033
Thr Trp Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val	
290 295 300	
tcc ttc ttc gcg ctg cct gca ggc atc ttg ggg tct ggg ttt gcc ctg	1081
Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu	
305 310 315	
aag gtt cag gag cag cac agg cag aag cac ttt gag aag agg cgg aac	1129
Lys Val Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn	
320 325 330	
ccg gca gca ggc ctg atc cag tcg gcc tgg aga ttc tac gcc acc aac	1177
Pro Ala Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn	
335 340 345 350	
ctc tcg cgc aca gac ctg cac tcc acg tgg cag tac tac gag cga acg	1225
Leu Ser Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr	
355 360 365	
gtc acc gtg ccc atg tac agt tcg caa act caa acc tac ggg gcc tcc	1273
Val Thr Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser	
370 375 380	
aga ctt atc ccc ccg ctg aac cag ctg gag ctg ctg agg aac ctc aag	1321
Arg Leu Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys	
385 390 395	
agt aaa tct gga ctc gct ttc agg aag gac ccc ccg ccg gag ccg tct	1369
Ser Lys Ser Gly Leu Ala Phe Arg Lys Asp Pro Pro Pro Glu Pro Ser	
400 405 410	
cca agt aaa ggc agc ccg tgc aga ggg ccc ctg tgt gga tgc tgc ccc	1417
Pro Ser Lys Gly Ser Pro Cys Arg Gly Pro Leu Cys Gly Cys Cys Pro	
415 420 425 430	
gga cgc tct agc cag aag gtc agt ttg aaa gat cgt gtc ttc tcc agc	1465
Gly Arg Ser Ser Gln Lys Val Ser Leu Lys Asp Arg Val Phe Ser Ser	
435 440 445	
ccc cga ggc gtg gct gcc aag ggg aag ggg tcc ccg cag gcc cag act	1513
Pro Arg Gly Val Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr	
450 455 460	
gtg agg cgg tca ccc agc gcc gac cag agc ctc gag gac agc ccc agc	1561
Val Arg Arg Ser Pro Ser Ala Asp Gln Ser Leu Glu Asp Ser Pro Ser	
465 470 475	

aag gtg ccc aag agc tgg agc ttc ggg gac cgc agc cgg gca cgc cag	1609
Lys Val Pro Lys Ser Trp Ser Phe Gly Asp Arg Ser Arg Ala Arg Gln	
480 485 490	
gct ttc cgc atc aag ggt gcc gcg tca cgg cag aac tca gaa gaa gca	1657
Ala Phe Arg Ile Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala	
495 500 505 510	
agc ctc ccc gga gag gac att gtg gat gac aag agc tgc ccc tgc gag	1705
Ser Leu Pro Gly Glu Asp Ile Val Asp Asp Lys Ser Cys Pro Cys Glu	
515 520 525	
ttt gtg acc gag gac ctg acc ccg ggc ctc aaa gtc agc atc aga gcc	1753
Phe Val Thr Glu Asp Leu Thr Pro Gly Leu Lys Val Ser Ile Arg Ala	
530 535 540	
gtg tgt gtc atg cgg ttc ctg gtg tcc aag cgg aag ttc aag gag agc	1801
Val Cys Val Met Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu Ser	
545 550 555	
ctg cgg ccc tac gac gtg atg gac gtc atc gag cag tac tca gcc ggc	1849
Leu Arg Pro Tyr Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala Gly	
560 565 570	
cac ctg gac atg ctg tcc cga att aag agc ctg cag tcc aga gtg gac	1897
His Leu Asp Met Leu Ser Arg Ile Lys Ser Leu Gln Ser Arg Val Asp	
575 580 585 590	
cag atc gtg ggg cgg ggc cca gcg atc acg gac aag gac cgc acc aag	1945
Gln Ile Val Gly Arg Gly Pro Ala Ile Thr Asp Lys Asp Arg Thr Lys	
595 600 605	
ggc ccg gcc gag gcg gag ctg ccc gag gac ccc agc atg atg gga cgg	1993
Gly Pro Ala Glu Ala Glu Leu Pro Glu Asp Pro Ser Met Met Gly Arg	
610 615 620	
ctc ggg aag gtg gag aag cag gtc ttg tcc atg gag aag aag ctg gac	2041
Leu Gly Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu Asp	
625 630 635	
ttc ctg gtg aat atc tac atg cag cgg atg ggc atc ccc ccg aca gag	2089
Phe Leu Val Asn Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu	
640 645 650	
acc gag gcc tac ttt ggg gcc aaa gag ccg gag ccg gcg ccg ccg tac	2137
Thr Glu Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr	
655 660 665 670	
cac agc ccg gaa gac agc cgg gag cat gtc gac agg cac ggc tgc att	2185
His Ser Pro Glu Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile	
675 680 685	
gtc aag atc gtg cgc tcc agc agc tcc acg ggc cag aag aac ttc tcg	2233
Val Lys Ile Val Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser	
690 695 700	

gcg ccc ccg gcc gcg ccc cct gtc cag tgt ccg ccc tcc acc tcc tgg 2281  
 Ala Pro Pro Ala Ala Pro Pro Val Gln Cys Pro Pro Ser Thr Ser Trp  
 705 710 715

cag cca cag agc cac ccg cgc cag ggc cac ggc acc tcc ccc gtg ggg 2329  
 Gln Pro Gln Ser His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly  
 720 725 730

gac cac ggc tcc ctg gtg cgc atc ccg ccg ccg cct gcc cac gag cgg 2377  
 Asp His Gly Ser Leu Val Arg Ile Pro Pro Pro Pro Ala His Glu Arg  
 735 740 745 750

tcg ctg tcc gcc tac ggc ggg ggc aac cgc gcc agc atg gag ttc ctg 2425  
 Ser Leu Ser Ala Tyr Gly Gly Gly Asn Arg Ala Ser Met Glu Phe Leu  
 755 760 765

cgg cag gag gac acc ccg ggc tgc agg ccc ccc gag ggg aac ctg cgg 2473  
 Arg Gln Glu Asp Thr Pro Gly Cys Arg Pro Pro Glu Gly Asn Leu Arg  
 770 775 780

gac agc gac acg tcc atc tcc atc ccg tcc gtg gac cac gag gag ctg 2521  
 Asp Ser Asp Thr Ser Ile Ser Ile Pro Ser Val Asp His Glu Glu Leu  
 785 790 795

gag cgt tcc ttc agc ggc ttc agc atc tcc cag tcc aag gag aac ctg 2569  
 Glu Arg Ser Phe Ser Gly Phe Ser Ile Ser Gln Ser Lys Glu Asn Leu  
 800 805 810

gat gct ctc aac agc tgc tac gcg gcc gtg gcg cct tgt gcc aaa gtc 2617  
 Asp Ala Leu Asn Ser Cys Tyr Ala Ala Val Ala Pro Cys Ala Lys Val  
 815 820 825 830

agg ccc tac att gcg gag gga gag tca gac acc gac tcc gac ctc tgt 2665  
 Arg Pro Tyr Ile Ala Glu Gly Glu Ser Asp Thr Asp Ser Asp Leu Cys  
 835 840 845

acc ccg tgc ggg ccc ccg cca cgc tgc gcc acc ggc gag ggt ccc ttt 2713  
 Thr Pro Cys Gly Pro Pro Pro Arg Ser Ala Thr Gly Glu Gly Pro Phe  
 850 855 860

ggt gac gtg ggc tgg gcc ggg ccc agg aag tgaggcggcg ctgggccagt 2763  
 Gly Asp Val Gly Trp Ala Gly Pro Arg Lys  
 865 870

ggacccgccc ggggccctcc tcagcacggt gcctccgagg ttttgaggcg ggaaccctct 2823

gggggcccttt tcttacagta actgagtgtg gcgggaaggg tggggccctgg agggggcccat 2883

gtgggctgaa ggatgggggc tcctggcagt gaccttttac aaaagttatt ttccaacagg 2943

ggctggaggg ctgggcaggg cctgtggctc caggagcagc gtgcaggagc aaggctgccc 3003

tgtccactct gctcaaggcc gcggccgaca tcagcccgggt gtgaagaggg gcggagtgat 3063

7

gacgggtggtt gcaacctggc aacaagcngg gggttgncca gccganccaa gggaagcaca 3123  
 naaggaagct gtnccctaag acctnccna aaggcggcct gtttggttaag actgcgcctt 3183  
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 <211> 872  
 <212> PRT  
 <213> Homo sapiens

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 20 25 30  
 Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro  
 35 40 45  
 Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala  
 50 55 60  
 Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
 65 70 75 80  
 Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His  
 85 90 95  
 Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
 100 105 110  
 Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile  
 115 120 125  
 Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
 130 135 140  
 Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
 145 150 155 160  
 Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu  
 165 170 175  
 Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe  
 180 185 190  
 Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met  
 195 200 205  
 Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val  
 210 215 220

Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe  
 225 230 235 240  
 Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly  
 245 250 255  
 Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu  
 260 265 270  
 Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp  
 275 280 285  
 Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe  
 290 295 300  
 Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val  
 305 310 315 320  
 Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala  
 325 330 335  
 Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser  
 340 345 350  
 Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr Val Thr  
 355 360 365  
 Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu  
 370 375 380  
 Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys  
 385 390 395 400  
 Ser Gly Leu Ala Phe Arg Lys Asp Pro Pro Pro Glu Pro Ser Pro Ser  
 405 410 415  
 Lys Gly Ser Pro Cys Arg Gly Pro Leu Cys Gly Cys Cys Pro Gly Arg  
 420 425 430  
 Ser Ser Gln Lys Val Ser Leu Lys Asp Arg Val Phe Ser Ser Pro Arg  
 435 440 445  
 Gly Val Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr Val Arg  
 450 455 460  
 Arg Ser Pro Ser Ala Asp Gln Ser Leu Glu Asp Ser Pro Ser Lys Val  
 465 470 475 480  
 Pro Lys Ser Trp Ser Phe Gly Asp Arg Ser Arg Ala Arg Gln Ala Phe  
 485 490 495  
 Arg Ile Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala Ser Leu  
 500 505 510



Pro Gly Glu Asp Ile Val Asp Asp Lys Ser Cys Pro Cys Glu Phe Val  
 515 520 525

Thr Glu Asp Leu Thr Pro Gly Leu Lys Val Ser Ile Arg Ala Val Cys  
 530 535 540

Val Met Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu Ser Leu Arg  
 545 550 555 560

Pro Tyr Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu  
 565 570 575

Asp Met Leu Ser Arg Ile Lys Ser Leu Gln Ser Arg Val Asp Gln Ile  
 580 585 590

Val Gly Arg Gly Pro Ala Ile Thr Asp Lys Asp Arg Thr Lys Gly Pro  
 595 600 605

Ala Glu Ala Glu Leu Pro Glu Asp Pro Ser Met Met Gly Arg Leu Gly  
 610 615 620

Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu Asp Phe Leu  
 625 630 635 640

Val Asn Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu Thr Glu  
 645 650 655

Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr His Ser  
 660 665 670

Pro Glu Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile Val Lys  
 675 680 685

Ile Val Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser Ala Pro  
 690 695 700

Pro Ala Ala Pro Pro Val Gln Cys Pro Pro Ser Thr Ser Trp Gln Pro  
 705 710 715 720

Gln Ser His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly Asp His  
 725 730 735

Gly Ser Leu Val Arg Ile Pro Pro Pro Pro Ala His Glu Arg Ser Leu  
 740 745 750

Ser Ala Tyr Gly Gly Gly Asn Arg Ala Ser Met Glu Phe Leu Arg Gln  
 755 760 765

Glu Asp Thr Pro Gly Cys Arg Pro Pro Glu Gly Asn Leu Arg Asp Ser  
 770 775 780

Asp Thr Ser Ile Ser Ile Pro Ser Val Asp His Glu Glu Leu Glu Arg  
 785 790 795 800

10

Ser Phe Ser Gly Phe Ser Ile Ser Gln Ser Lys Glu Asn Leu Asp Ala  
805 810 815

Leu Asn Ser Cys Tyr Ala Ala Val Ala Pro Cys Ala Lys Val Arg Pro  
820 825 830

Tyr Ile Ala Glu Gly Glu Ser Asp Thr Asp Ser Asp Leu Cys Thr Pro  
835 840 845

Cys Gly Pro Pro Pro Arg Ser Ala Thr Gly Glu Gly Pro Phe Gly Asp  
850 855 860

Val Gly Trp Ala Gly Pro Arg Lys  
865 870

<210> 3  
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<212> PRT  
<213> Homo sapiens

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Asp Gln Ala Ala Pro Ser Asp Glu Gln Gln Glu Ala Gly Ser Ser Ser  
35 40 45

Ala Ile Gly Gln Glu Ser Arg Lys Thr Val Val Phe Gln Glu Pro Asp  
50 55 60

Ile Gly Phe Pro Ser Glu His Asp Gln Leu Thr Thr Leu His Asp Ser  
65 70 75 80

Glu Glu Gly Asn Arg Lys Met Ser Leu Val Gly Lys Pro Leu Thr Tyr  
85 90 95

Lys Asn Tyr Arg Thr Asp Gln Arg Phe Arg Arg Met Gln Asn Lys Met  
100 105 110

His Asn Phe Leu Glu Arg Pro Arg Gly Trp Lys Ala Ala Thr Tyr His  
115 120 125

Leu Ala Val Leu Phe Met Val Leu Met Cys Leu Ala Leu Ser Val Phe  
130 135 140

Ser Thr Met Pro Asp Phe Glu Val Asn Ala Thr Ile Val Leu Tyr Tyr  
145 150 155 160

Leu Glu Ile Val Phe Val Ile Trp Leu Ala Thr Glu Tyr Ile Cys Arg  
165 170 175

Val Trp Ser Ala Gly Cys Arg Ser Arg Tyr Arg Gly Ile Ser Gly Arg  
 180 185 190  
 Ile Arg Phe Ala Thr Ser Ala Tyr Cys Val Ile Asp Ile Ile Val Ile  
 195 200 205  
 Leu Ala Ser Ile Thr Val Leu Cys Ile Gly Ala Thr Gly Gln Val Phe  
 210 215 220  
 Ala Ala Ser Ala Ile Arg Gly Leu Arg Phe Phe Gln Leu Arg Met Leu  
 225 230 235 240  
 Arg Ile Asp Arg Arg Ala Gly Thr Trp Lys Leu Leu Gly Ser Val Val  
 245 250 255  
 Trp Ala His Arg Gln Glu Leu Leu Thr Thr Val Tyr Ile Gly Phe Leu  
 260 265 270  
 Gly Leu Ile Phe Ser Ser Phe Leu Val Tyr Leu Cys Glu Lys Asn Thr  
 275 280 285  
 Asn Asp Lys Tyr Gln Thr Phe Ala Asp Ala Leu Trp Trp Gly Val Ile  
 290 295 300  
 Thr Leu Ser Thr Val Gly Tyr Gly Asp Lys Thr Pro Glu Thr Trp Pro  
 305 310 315 320  
 Gly Lys Ile Ile Ala Ala Phe Cys Ala Leu Leu Gly Ile Ser Phe Phe  
 325 330 335  
 Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val Gln  
 340 345 350  
 Gln His Gln Arg Gln Lys His Leu Ile Arg Arg Arg Val Pro Ala Ala  
 355 360 365  
 Lys Leu Ile Gln Cys Leu Trp Arg His Tyr Ser Ala Ala Pro Glu Ser  
 370 375 380  
 Thr Ser Leu Ala Thr Trp Lys Ile His Leu Ala Arg Glu Leu Pro Pro  
 385 390 395 400  
 Ile Val Lys Leu Thr Pro Leu Gly Ser Asn Asn Ala Thr Gly Leu Ile  
 405 410 415  
 Asn Arg Leu Arg Gln Ser Thr Lys Arg Thr Pro Asn Leu Asn Asn Gln  
 420 425 430  
 Asn Leu Ala Val Asn Ser Gln Ala Thr Ser Lys Asn Leu Ser Val Pro  
 435 440 445  
 Arg Val Arg Val His Asp Thr Ile Ser Leu Val Ser Thr Ser Asp Ile  
 450 455 460

Ser Glu Ile Glu Gln Leu Gly Ala Leu Gly Phe Ser Leu Gly Trp Lys  
 465 470 475 480  
 Ser Lys Ser Lys Tyr Gly Gly Ser Lys Lys Ala Thr Asp Asp Ser Val  
 485 490 495  
 Leu Gln Ser Arg Met Leu Ala Pro Ser Asn Ala His Leu Asp Asp Met  
 500 505 510  
 Arg Arg Arg Ser Arg Arg Ser Ala Ser Leu Cys Arg Val Val Asn Thr  
 515 520 525  
 Gly Gln His Leu Arg Pro Leu Gln Pro Arg Ser Thr Leu Ser Asp Ser  
 530 535 540  
 Asp Val Ile Gly Asp Tyr Ser Leu Met Met Ala Pro Ile Tyr Gln Trp  
 545 550 555 560  
 Cys Glu Gln Met Val Gln Arg Asn Ser Thr Pro Gly Glu Asp Gly Val  
 565 570 575  
 Trp Ser Gln Leu Ser Gln Leu Ser Gln Leu Thr Thr Cys Ala Thr Arg  
 580 585 590  
 Arg Thr Glu Asp Ile Ser Asp Gly Asp Glu Glu Glu Ala Val Gly Tyr  
 595 600 605  
 Gln Pro Gln Thr Ile Glu Glu Phe Thr Pro Ala Leu Lys Asn Cys Val  
 610 615 620  
 Arg Ala Ile Arg Arg Ile Gln Leu Leu Val Ala Arg Lys Lys Phe Lys  
 625 630 635 640  
 Glu Ala Leu Lys Pro Tyr Asp Val Lys Asp Val Ile Glu Gln Tyr Ser  
 645 650 655  
 Ala Gly His Val Asp Leu Gln Ser Arg Val Lys Thr Val Gln Ala Lys  
 660 665 670  
 Leu Asp Phe Ile Cys Gly Lys Asn Ile Glu Lys Ile Glu Pro Lys Ile  
 675 680 685  
 Ser Met Phe Thr Arg Ile Ala Thr Leu Glu Thr Thr Val Gly Lys Met  
 690 695 700  
 Asp Lys Lys Leu Asp Leu Met Val Glu Met Leu Met Gly Arg Gln Ala  
 705 710 715 720  
 Ser Gln Arg Val Phe Ser Gln Asn Thr Ser Pro Arg Gly Glu Phe Ser  
 725 730 735  
 Glu Pro Thr Ser Ala Arg Gln Asp Leu Thr Arg Ser Arg Arg Ser Met  
 740 745 750

13

Val Ser Thr Asp Met Glu Met Tyr Thr Ala Arg Ser His Ser Pro Gly  
 755 760 765

Tyr His Gly Asp Ala Arg Pro Ile Ile Ala Gln Ile Asp Ala Asp Asp  
 770 775 780

Asp Asp Glu Asp Glu Asn Val Phe Asp Asp Ser Thr Pro Leu Asn Asn  
 785 790 795 800

Gly Pro Gly Thr Ser Ser Cys  
 805

<210> 4  
 <211> 677  
 <212> PRT  
 <213> Homo sapiens

<400> 4  
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Trp Gly Arg Leu Pro Gly Ala Arg Arg Gly Ser Ala Gly Leu Ala Lys  
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Lys Cys Pro Phe Ser Leu Glu Leu Ala Glu Gly Gly Pro Ala Gly Gly  
 35 40 45

Ala Leu Tyr Ala Pro Ile Ala Pro Gly Ala Pro Gly Pro Ala Pro Pro  
 50 55 60

Ala Ser Pro Ala Ala Pro Ala Ala Pro Pro Val Ala Ser Asp Leu Gly  
 65 70 75 80

Pro Arg Pro Pro Val Ser Asp Leu Pro Arg Val Ser Ile Tyr Ser Thr  
 85 90 95

Arg Arg Pro Val Leu Ala Arg Thr His Val Gln Gly Arg Val Tyr Asn  
 100 105 110

Phe Leu Glu Arg Pro Thr Gly Trp Lys Cys Phe Val Tyr His Phe Ala  
 115 120 125

Val Phe Leu Ile Val Leu Val Cys Leu Ile Phe Ser Val Leu Ser Thr  
 130 135 140

Ile Glu Gln Tyr Ala Ala Leu Ala Thr Gly Thr Leu Phe Trp Met Glu  
 145 150 155 160

Ile Val Leu Val Val Phe Phe Gly Thr Glu Tyr Val Val Arg Leu Trp  
 165 170 175

Ser Ala Gly Cys Arg Ser Lys Tyr Val Gly Leu Trp Gly Arg Leu Arg  
 180 185 190

Phe Phe Ala Arg Lys Pro Ile Ser Ile Ile Asp Leu Ile Val Val Val  
 195 200 205  
 Ala Ser Met Val Val Leu Cys Val Gly Ser Lys Gly Gln Val Phe Ala  
 210 215 220  
 Thr Ser Ala Ile Arg Gly Ile Arg Phe Leu Gln Ile Leu Arg Met Leu  
 225 230 235 240  
 His Val Asp Arg Gln Gly Gly Thr Trp Arg Leu Leu Gly Ser Val Val  
 245 250 255  
 Phe Ile His Arg Gln Glu Leu Ile Thr Thr Leu Tyr Ile Gly Phe Leu  
 260 265 270  
 Gly Leu Ile Phe Ser Ser Tyr Phe Val Tyr Leu Ala Glu Lys Asp Ala  
 275 280 285  
 Val Asn Glu Ser Gly Arg Val Glu Phe Gly Ser Tyr Ala Asp Ala Leu  
 290 295 300  
 Trp Trp Gly Val Val Thr Val Thr Thr Ile Gly Tyr Gly Asp Lys Val  
 305 310 315 320  
 Pro Gln Thr Trp Val Gly Lys Thr Ile Ala Ser Cys Phe Ser Val Phe  
 325 330 335  
 Ala Ile Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe  
 340 345 350  
 Ala Leu Lys Val Gln Gln Lys Gln Arg Gln Lys His Phe Asn Arg Gln  
 355 360 365  
 Ile Pro Ala Ala Ala Ser Leu Ile Gln Thr Ala Trp Arg Cys Tyr Ala  
 370 375 380  
 Ala Glu Asn Pro Asp Ser Ser Thr Trp Lys Ile Tyr Ile Arg Lys Ala  
 385 390 395 400  
 Pro Arg Ser His Thr Leu Leu Ser Pro Ser Pro Lys Pro Lys Lys Ser  
 405 410 415  
 Val Val Val Lys Lys Lys Lys Phe Lys Leu Asp Lys Asp Asn Gly Val  
 420 425 430  
 Thr Pro Gly Glu Lys Met Leu Thr Val Pro His Ile Thr Cys Asp Pro  
 435 440 445  
 Pro Glu Glu Arg Arg Leu Asp His Phe Ser Val Asp Gly Tyr Asp Ser  
 450 455 460  
 Ser Val Arg Lys Ser Pro Thr Leu Leu Glu Val Ser Met Pro His Phe  
 465 470 475 480

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<210> 5
<211> 12
<212> DNA
<213> Homo sapiens

<220>
<221> mutation
<222> (5)
<223> The mutation from G to A occurs at this site in
      kindred K3933.

<220>
<221> intron
<222> (1)..(5)
```

16

<220>  
 <221> exon  
 <222> (6)..(11)

<400> 5  
 tgcag tgt cat g

12

<210> 6  
 <211> 2914  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (19)..(2634)

<220>  
 <221> allele  
 <222> (840)  
 <223> The polymorphism of a T to a C at this position  
 has appeared in one individual.

<220>  
 <221> mutation  
 <222> (947)  
 <223> The missense mutation from a G to a T occurs at  
 this position in a BFNC family.

<220>  
 <221> allele  
 <222> (678)  
 <223> This position is polymorphic for C or T.

<220>  
 <221> allele  
 <222> (750)  
 <223> This position is polymorphic for T or C.

<220>  
 <221> allele  
 <222> (1089)  
 <223> This position is polymorphic for G or C.

<220>  
 <221> allele  
 <222> (2598)  
 <223> This position is polymorphic for T or C.

<400> 6  
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 Met Gly Leu Lys Ala Arg Arg Ala Ala Gly Ala  
 1 5 10



17

gct ggc ggc ggc ggc gac ggg ggc ggc gga ggc ggc ggg gcg gct aac	99
Ala Gly Gly Gly Gly Asp Gly Gly Gly Gly Gly Gly Ala Ala Asn	
15 20 25	
cca gcc gga ggg gac gcg gcg gcg gcc ggc gac gag gag cgg aaa gtg	147
Pro Ala Gly Gly Asp Ala Ala Ala Ala Gly Asp Glu Glu Arg Lys Val	
30 35 40	
ggg ctg gcg ccc ggc gac gtg gag caa gtc acc ttg gcg ctc ggg gcc	195
Gly Leu Ala Pro Gly Asp Val Glu Gln Val Thr Leu Ala Leu Gly Ala	
45 50 55	
gga gcc gac aaa gac ggg acc ctg ctg ctg gag ggc ggc ggc cgc gac	243
Gly Ala Asp Lys Asp Gly Thr Leu Leu Leu Glu Gly Gly Gly Arg Asp	
60 65 70 75	
gag ggg cag cgg agg acc ccg cag ggc atc ggg ctc ctg gcc aag acc	291
Glu Gly Gln Arg Arg Thr Pro Gln Gly Ile Gly Leu Leu Ala Lys Thr	
80 85 90	
ccg ctg agc cgc cca gtc aag aga aac aac gcc aag tac cgg cgc atc	339
Pro Leu Ser Arg Pro Val Lys Arg Asn Asn Ala Lys Tyr Arg Arg Ile	
95 100 105	
caa act ttg atc tac gac gcc ctg gag aga ccg cgg ggc tgg gcg ctg	387
Gln Thr Leu Ile Tyr Asp Ala Leu Glu Arg Pro Arg Gly Trp Ala Leu	
110 115 120	
ctt tac cac gcg ttg gtg ttc ctg att gtc ctg ggg tgc ttg att ctg	435
Leu Tyr His Ala Leu Val Phe Leu Ile Val Leu Gly Cys Leu Ile Leu	
125 130 135	
gct gtc ctg acc aca ttc aag gag tat gag act gtc tcg gga gac tgg	483
Ala Val Leu Thr Thr Phe Lys Glu Tyr Glu Thr Val Ser Gly Asp Trp	
140 145 150 155	
ctt ctg tta ctg gag aca ttt gct att ttc atc ttt gga gcc gag ttt	531
Leu Leu Leu Leu Glu Thr Phe Ala Ile Phe Ile Phe Gly Ala Glu Phe	
160 165 170	
gct ttg agg atc tgg gct gct gga tgt tgc tgc cga tac aaa ggc tgg	579
Ala Leu Arg Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Lys Gly Trp	
175 180 185	
cgg ggc cga ctg aag ttt gcc agg aag ccc ctg tgc atg ttg gac atc	627
Arg Gly Arg Leu Lys Phe Ala Arg Lys Pro Leu Cys Met Leu Asp Ile	
190 195 200	
ttt gtg ctg att gcc tct gtg cca gtg gtt gct gtg gga aac caa ggc	675
Phe Val Leu Ile Ala Ser Val Pro Val Val Ala Val Gly Asn Gln Gly	
205 210 215	
aat gtt ctg gcc acc tcc ctg cga agc ctg cgc ttc ctg cag atc ctg	723
Asn Val Leu Ala Thr Ser Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu	
220 225 230 235	

cgc atg ctg cgg atg gac cgg aga ggt ggc acc tgg aag ctt ctg ggc	771
Arg Met Leu Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly	
240 245 250	
tca gcc atc tgt gcc cac agc aaa gaa ctc atc acg gcc tgg tac atc	819
Ser Ala Ile Cys Ala His Ser Lys Glu Leu Ile Thr Ala Trp Tyr Ile	
255 260 265	
ggg ttc ctg aca ctc atc ctt tct tca ttt ctt gtc tac ctg gtt gag	867
Gly Phe Leu Thr Leu Ile Leu Ser Ser Phe Leu Val Tyr Leu Val Glu	
270 275 280	
aaa gac gtc cca gag gtg gat gca caa gga gag gag atg aaa gag gag	915
Lys Asp Val Pro Glu Val Asp Ala Gln Gly Glu Glu Met Lys Glu Glu	
285 290 295	
ttt gag acc tat gca gat gcc ctg tgg tgg ggc ctg atc aca ctg gcc	963
Phe Glu Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu Ile Thr Leu Ala	
300 305 310 315	
acc att ggc tat gga gac aag aca ccc aaa acg tgg gaa ggc cgt ctg	1011
Thr Ile Gly Tyr Gly Asp Lys Thr Pro Lys Thr Trp Glu Gly Arg Leu	
320 325 330	
att gcc gcc acc ttt tcc tta att ggc gtc tcc ttt ttt gcc ctt cca	1059
Ile Ala Ala Thr Phe Ser Leu Ile Gly Val Ser Phe Phe Ala Leu Pro	
335 340 345	
gcg ggc atc ctg ggg tcc ggg ctg gcc ctc aag gtg cag gag caa cac	1107
Ala Gly Ile Leu Gly Ser Gly Leu Ala Leu Lys Val Gln Glu Gln His	
350 355 360	
cgt cag aag cac ttt gag aaa agg agg aag cca gct gct gag ctc att	1155
Arg Gln Lys His Phe Glu Lys Arg Arg Lys Pro Ala Ala Glu Leu Ile	
365 370 375	
cag gct gcc tgg agg tat tat gct acc aac ccc aac agg att gac ctg	1203
Gln Ala Ala Trp Arg Tyr Tyr Ala Thr Asn Pro Asn Arg Ile Asp Leu	
380 385 390 395	
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Val Ala Thr Trp Arg Phe Tyr Glu Ser Val Val Ser Phe Pro Phe Phe	
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Arg Lys Glu Gln Leu Glu Ala Ala Ser Ser Gln Lys Leu Gly Leu Leu	
415 420 425	
gat cgg gtt cgc ctt tct aat cct cgt ggt agc aat act aaa gga aag	1347
Asp Arg Val Arg Leu Ser Asn Pro Arg Gly Ser Asn Thr Lys Gly Lys	
430 435 440	

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Leu Phe Thr Pro Leu Asn Val Asp Ala Ile Glu Glu Ser Pro Ser Lys	
445 450 455	
gaa cca aag cct gtt ggc tta aac aat aaa gag cgt ttc cgc acg gcc	1443
Glu Pro Lys Pro Val Gly Leu Asn Asn Lys Glu Arg Phe Arg Thr Ala	
460 465 470 475	
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Phe Arg Met Lys Ala Tyr Ala Phe Trp Gln Ser Ser Glu Asp Ala Gly	
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Thr Gly Asp Pro Met Ala Glu Asp Arg Gly Tyr Gly Asn Asp Phe Pro	
495 500 505	
atc gaa gac atg atc ccc acc ctg aag gcc gcc atc cga gcc gtc aga	1587
Ile Glu Asp Met Ile Pro Thr Leu Lys Ala Ala Ile Arg Ala Val Arg	
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Ile Leu Gln Phe Arg Leu Tyr Lys Lys Lys Phe Lys Glu Thr Leu Arg	
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cct tac gat gtg aag gat gtg att gag cag tat tct gcc ggg cat ctc	1683
Pro Tyr Asp Val Lys Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu	
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Asp Met Leu Ser Arg Ile Lys Tyr Leu Gln Thr Arg Ile Asp Met Ile	
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Phe Thr Pro Gly Pro Pro Ser Thr Pro Lys His Lys Lys Ser Gln Lys	
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Gly Ser Ala Phe Thr Phe Pro Ser Gln Gln Ser Pro Arg Asn Glu Pro	
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Tyr Val Ala Arg Pro Ser Thr Ser Glu Ile Glu Asp Gln Ser Met Met	
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Gly Lys Phe Val Lys Val Glu Arg Gln Val Gln Asp Met Gly Lys Lys	
620 625 630 635	
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Leu Asp Phe Leu Val Asp Met His Met Gln His Met Glu Arg Leu Gln	
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Val Gln Val Thr Glu Tyr Tyr Pro Thr Lys Gly Thr Ser Ser Pro Ala	
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cac cag gtg acc att gac aaa gtc agc ccc tat ggg ttt ttt gca cat His Gln Val Thr Ile Asp Lys Val Ser Pro Tyr Gly Phe Phe Ala His 700 705 710 715	2163
gac cct gtg aac ctg ccc cga ggg gga ccc agt tct gga aag gtt cag Asp Pro Val Asn Leu Pro Arg Gly Gly Pro Ser Ser Gly Lys Val Gln 720 725 730	2211
gca act cct cct tcc tca gca aca acg tat gtg gag agg ccc acg gtc Ala Thr Pro Ser Ser Ala Thr Tyr Val Glu Arg Pro Thr Val 735 740 745	2259
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cgt agc atc acg cga gac agt gac aca cct ctg tcc ctg atg tcg gtc Arg Ser Ile Thr Arg Asp Ser Asp Thr Pro Leu Ser Leu Met Ser Val 780 785 790 795	2403
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ccc ttc acg ccc agc ggc tcc atg cct ctg tcg tcc aca ggg gat ggg Pro Phe Thr Pro Ser Gly Ser Met Pro Leu Ser Ser Thr Gly Asp Gly 845 850 855	2595
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21

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 35 40 45  
 Asp Val Glu Gln Val Thr Leu Ala Leu Gly Ala Gly Ala Asp Lys Asp  
 50 55 60  
 Gly Thr Leu Leu Leu Glu Gly Gly Gly Arg Asp Glu Gly Gln Arg Arg  
 65 70 75 80  
 Thr Pro Gln Gly Ile Gly Leu Leu Ala Lys Thr Pro Leu Ser Arg Pro  
 85 90 95  
 Val Lys Arg Asn Asn Ala Lys Tyr Arg Arg Ile Gln Thr Leu Ile Tyr  
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 Asp Ala Leu Glu Arg Pro Arg Gly Trp Ala Leu Leu Tyr His Ala Leu  
 115 120 125  
 Val Phe Leu Ile Val Leu Gly Cys Leu Ile Leu Ala Val Leu Thr Thr  
 130 135 140  
 Phe Lys Glu Tyr Glu Thr Val Ser Gly Asp Trp Leu Leu Leu Leu Glu  
 145 150 155 160  
 Thr Phe Ala Ile Phe Ile Phe Gly Ala Glu Phe Ala Leu Arg Ile Trp  
 165 170 175  
 Ala Ala Gly Cys Cys Cys Arg Tyr Lys Gly Trp Arg Gly Arg Leu Lys  
 180 185 190  
 Phe Ala Arg Lys Pro Leu Cys Met Leu Asp Ile Phe Val Leu Ile Ala  
 195 200 205

Ser Val Pro Val Val Ala Val Gly Asn Gln Gly Asn Val Leu Ala Thr  
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 Ser Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met Leu Arg Met  
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 Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Ala Ile Cys Ala  
 245 250 255  
 His Ser Lys Glu Leu Ile Thr Ala Trp Tyr Ile Gly Phe Leu Thr Leu  
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 Ile Leu Ser Ser Phe Leu Val Tyr Leu Val Glu Lys Asp Val Pro Glu  
 275 280 285  
 Val Asp Ala Gln Gly Glu Glu Met Lys Glu Glu Phe Glu Thr Tyr Ala  
 290 295 300  
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 305 310 315 320  
 Asp Lys Thr Pro Lys Thr Trp Glu Gly Arg Leu Ile Ala Ala Thr Phe  
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 Ser Leu Ile Gly Val Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly  
 340 345 350  
 Ser Gly Leu Ala Leu Lys Val Gln Glu Gln His Arg Gln Lys His Phe  
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 Glu Lys Arg Arg Lys Pro Ala Ala Glu Leu Ile Gln Ala Ala Trp Arg  
 370 375 380  
 Tyr Tyr Ala Thr Asn Pro Asn Arg Ile Asp Leu Val Ala Thr Trp Arg  
 385 390 395 400  
 Phe Tyr Glu Ser Val Val Ser Phe Pro Phe Phe Arg Lys Glu Gln Leu  
 405 410 415  
 Glu Ala Ala Ser Ser Gln Lys Leu Gly Leu Leu Asp Arg Val Arg Leu  
 420 425 430  
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 435 440 445  
 Asn Val Asp Ala Ile Glu Glu Ser Pro Ser Lys Glu Pro Lys Pro Val  
 450 455 460  
 Gly Leu Asn Asn Lys Glu Arg Phe Arg Thr Ala Phe Arg Met Lys Ala  
 465 470 475 480  
 Tyr Ala Phe Trp Gln Ser Ser Glu Asp Ala Gly Thr Gly Asp Pro Met  
 485 490 495

Ala Glu Asp Arg Gly Tyr Gly Asn Asp Phe Pro Ile Glu Asp Met Ile  
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Pro Thr Leu Lys Ala Ala Ile Arg Ala Val Arg Ile Leu Gln Phe Arg  
 515 520 525

Leu Tyr Lys Lys Lys Phe Lys Glu Thr Leu Arg Pro Tyr Asp Val Lys  
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 545 550 555 560

Ile Lys Tyr Leu Gln Thr Arg Ile Asp Met Ile Phe Thr Pro Gly Pro  
 565 570 575

Pro Ser Thr Pro Lys His Lys Lys Ser Gln Lys Gly Ser Ala Phe Thr  
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Phe Pro Ser Gln Gln Ser Pro Arg Asn Glu Pro Tyr Val Ala Arg Pro  
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Ser Thr Ser Glu Ile Glu Asp Gln Ser Met Met Gly Lys Phe Val Lys  
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Val Glu Arg Gln Val Gln Asp Met Gly Lys Lys Leu Asp Phe Leu Val  
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Asp Met His Met Gln His Met Glu Arg Leu Gln Val Gln Val Thr Glu  
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Tyr Tyr Pro Thr Lys Gly Thr Ser Ser Pro Ala Glu Ala Glu Lys Lys  
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Glu Asp Asn Arg Tyr Ser Asp Leu Lys Thr Ile Ile Cys Asn Tyr Ser  
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Glu Thr Gly Pro Pro Glu Pro Pro Tyr Ser Phe His Gln Val Thr Ile  
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Asp Lys Val Ser Pro Tyr Gly Phe Phe Ala His Asp Pro Val Asn Leu  
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Pro Arg Gly Gly Pro Ser Ser Gly Lys Val Gln Ala Thr Pro Pro Ser  
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Ser Ala Thr Thr Tyr Val Glu Arg Pro Thr Val Leu Pro Ile Leu Thr  
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Leu Leu Asp Ser Arg Val Ser Cys His Ser Gln Ala Asp Leu Gln Gly  
 755 760 765

Pro Tyr Ser Asp Arg Ile Ser Pro Arg Gln Arg Arg Ser Ile Thr Arg  
 770 775 780

24

Asp Ser Asp Thr Pro Leu Ser Leu Met Ser Val Asn His Glu Glu Leu  
785 790 795 800

Glu Arg Ser Pro Ser Gly Phe Ser Ile Ser Gln Asp Arg Asp Asp Tyr  
805 810 815

Val Phe Gly Pro Asn Gly Gly Ser Ser Trp Met Arg Glu Lys Arg Tyr  
820 825 830

Leu Ala Glu Gly Glu Thr Asp Thr Asp Thr Asp Pro Phe Thr Pro Ser  
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identity.

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<212> DNA  
<213> Homo sapiens

<400> 86  
cttcagcatc tcccaggaca gag

23

<210> 87  
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<212> DNA  
<213> Homo sapiens

<400> 87  
aaggaggggt cagccagtga cct

23

&lt;210&gt; 88

&lt;211&gt; 2273

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(2271)

&lt;400&gt; 88

atg gtg cag aag tcg cgc aac ggt ggc gtg tac ccc ggc acc agc ggg	48
Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Thr Ser Gly	
1 5 10 15	
 gaa aan aan ctc aag gtg ggc ttc gtg ggg ctg gac ccc ggc gng ccc	96
Glu Xaa Xaa Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Xaa Pro	
20 25 30	
 gan tcc aca cgc gac ggc ncn cta ctc atc gcg ggc tcc gag gcc ccc	144
Xaa Ser Thr Arg Asp Gly Xaa Leu Leu Ile Ala Gly Ser Glu Ala Pro	
35 40 45	
 aag cgc ggc anc ntn ttg agc aag ccg cgg acg ggc ggc gcg gga ncc	192
Lys Arg Gly Xaa Xaa Leu Ser Lys Pro Arg Thr Gly Gly Ala Gly Xaa	
50 55 60	
 ggg aag ccc ccn aan cgc aac gcc ttc tac cgc aag ctg cag aat ttc	240
Gly Lys Pro Xaa Xaa Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe	
65 70 75 80	
 ctc tac aac gtg cta gag cgg ccc cgc ggc tng gcg ttc atc tac cac	288
Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Xaa Ala Phe Ile Tyr His	
85 90 95	
 gcc tac gtg ttc ctc ctg gtt ttc tcc tgc ctt gtg ctt tct gtg ttt	336
Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe	
100 105 110	
 tcc acc atc aag gag tac gag aag agc tct gag ggg gcc ctc tac atc	384
Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile	
115 120 125	
 ttg gaa atc gtg act atc gtg gta ttc ggt gtt gag tac ttt gtg agg	432
Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg	
130 135 140	
 atc tgg gct gca ggc tgc tgt tgc cgg tat cga ggc tgg agg ggc agg	480
Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg	
145 150 155 160	
 ctc aag ttt gcc agg aag ccg ttc tgt gtg att gat atc atg gtg ctg	528
Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu	
165 170 175	

39

att gcc tcc att gct gtg ctg gct gct ggt tcc cag ggc aat gtc ttt	576
Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe	
180 185 190	
gcc aca tct gcg ctt cgg agc ttg cgg ttc ttg caa atc ttg cgg atg	624
Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met	
195 200 205	
atc cgt atg gac cgg agg ggt ggc acc tgg aag ctc ttg gga tcg gta	672
Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val	
210 215 220	
gtc tac gct cac agc aag gag ctg gtg act gcc tgg tac att ggc ttc	720
Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe	
225 230 235 240	
ctc tgc ctc atc ctg gcc tca ttt ctg gtg tac ttg gca gaa aag ggt	768
Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly	
245 250 255	
gag aat gac cac ttt gac acc tac gca gat gca ctc tgg tgg ggt ctg	816
Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu	
260 265 270	
atc acc ctg acg acc att ggc tac ggg gac aag tac cct cag acc tgg	864
Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp	
275 280 285	
aac ggg agg ctg ctg gca gcg acc ttt acc ctc att ggt gtc tcg ttc	912
Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe	
290 295 300	
ttt gct ctt ccg gct ggc att ttg gga tcc ggc ttt gcc ctg aaa gtc	960
Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val	
305 310 315 320	
caa gag cag cat cgg caa aaa cac ttt gag aaa cgg cgg aac cct gcg	1008
Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala	
325 330 335	
gca ggt ctg atc cag tct gcc tgg aga ttc tat gct act aac ctc tca	1056
Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser	
340 345 350	
cgc acc gac ctg cac tcc acg tgg cag tac tac gag cgn aca gtc act	1104
Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Xaa Thr Val Thr	
355 360 365	
gtc ccc atg tac agc tca caa act caa acc tat ggg gcc tcc aga ctc	1152
Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu	
370 375 380	
atc cca cct ctg aac cag ctg gag ctg ctg agg aat ctc aag agc aaa	1200
Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys	
385 390 395 400	

tct gga ctc acc ttc agg aag gag cca cag cca gag cca tca cca agc	1248
Ser Gly Leu Thr Phe Arg Lys Glu Pro Gln Pro Glu Pro Ser Pro Ser	
405 410 415	
ccc cga ggc atg gct gcc aag gga aag ggg tct ccc cag gcc cag acg	1296
Pro Arg Gly Met Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr	
420 425 430	
gtc cgg cgg tcc ccc agt gcg gat cag agt ctt gat gac agc ccg agc	1344
Val Arg Arg Ser Pro Ser Ala Asp Gln Ser Leu Asp Asp Ser Pro Ser	
435 440 445	
aag gtg ccc aag agc tgg agc ttt ggt gac cgc agc cgc aca cgc cag	1392
Lys Val Pro Lys Ser Trp Ser Phe Gly Asp Arg Ser Arg Thr Arg Gln	
450 455 460	
gct ttc cgc atc aag ggt gct gca tcc cgg cag aat tca gaa gaa gca	1440
Ala Phe Arg Ile Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala	
465 470 475 480	
agc ctc cct ggg gag gac atc gta gag gac aac aag agc tgt aac tgc	1488
Ser Leu Pro Gly Glu Asp Ile Val Glu Asp Asn Lys Ser Cys Asn Cys	
485 490 495	
gag ttt gtg act gaa gat ctt acc cct ggc ctc aaa gty agc atc aga	1536
Glu Phe Val Thr Glu Asp Leu Thr Pro Gly Leu Lys Xaa Ser Ile Arg	
500 505 510	
gcc gtg tgt gty atg cgg ttc ttg gta tct aag cga aag ttc aaa gag	1584
Ala Val Cys Xaa Met Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu	
515 520 525	
agt ctg cgc cca tat gat gtg atg gac gtc atc gaa cag tac tcg gct	1632
Ser Leu Arg Pro Tyr Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala	
530 535 540	
gga cac ttg gat atg ttg tcc cgc atc aag agc ctg cag acc aga gtg	1680
Gly His Leu Asp Met Leu Ser Arg Ile Lys Ser Leu Gln Thr Arg Val	
545 550 555 560	
gac cag att gtg ggg cgg ggc cca aca ata acg gat aag gat cgc acc	1728
Asp Gln Ile Val Gly Arg Gly Pro Thr Ile Thr Asp Lys Asp Arg Thr	
565 570 575	
aaa ggc cca gcg gaa acg gag ctg ccc gaa gac ccc agc atg atg gga	1776
Lys Gly Pro Ala Glu Thr Glu Leu Pro Glu Asp Pro Ser Met Met Gly	
580 585 590	
cgg ctt ggg aag gtg gag aaa cag gtc ttg tcc atg gaa aag aag ctc	1824
Arg Leu Gly Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu	
595 600 605	



41

gac ttc ttg gtg agc atc tat aca cag aga atg ggc atc caa cca gca 1872  
 Asp Phe Leu Val Ser Ile Tyr Thr Gln Arg Met Gly Ile Gln Pro Ala  
 610 615 620

gag aca gag gcc tat ttt ggg gcc aag gag cct gag ccg gca cca ccc 1920  
 Glu Thr Glu Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro  
 625 630 635 640

tac cac agc cca gag gac agc cgt gac cat gca gac aag cat ggc tgt 1968  
 Tyr His Ser Pro Glu Asp Ser Arg Asp His Ala Asp Lys His Gly Cys  
 645 650 655

atc att aag atc gtc cgc tcc acc agc tct acg ggc cag agg aac tac 2016  
 Ile Ile Lys Ile Val Arg Ser Thr Ser Ser Thr Gly Gln Arg Asn Tyr  
 660 665 670

gca gca ccc cca gcc atc ccc cct gcc cag tgt cct ccc tcc acc tcg 2064  
 Ala Ala Pro Pro Ala Ile Pro Pro Ala Gln Cys Pro Pro Ser Thr Ser  
 675 680 685

tgg cgg cag agc cac cag cgc cat ggc acc tcc cct gtg gga gac cat 2112  
 Trp Arg Gln Ser His Gln Arg His Gly Thr Ser Pro Val Gly Asp His  
 690 695 700

ggc tca ctg gta cgc atc cca cca ctc cct gca cac gag cgg tcg ctg 2160  
 Gly Ser Leu Val Arg Ile Pro Pro Leu Pro Ala His Glu Arg Ser Leu  
 705 710 715 720

tct gcc tac ggt ggg ggc aac aga gcc agt acc gag ttc ttg agg ctg 2208  
 Ser Ala Tyr Gly Gly Asn Arg Ala Ser Thr Glu Phe Leu Arg Leu  
 725 730 735

gag ggc acc cca gcc tgc agg ccc tct gag gct gct gcg gga cag cga 2256  
 Glu Gly Thr Pro Ala Cys Arg Pro Ser Glu Ala Ala Ala Gly Gln Arg  
 740 745 750

cac gtc cat ctc cat cg 2273  
 His Val His Leu His  
 755

&lt;210&gt; 89

&lt;211&gt; 757

&lt;212&gt; PRT

&lt;213&gt; Mus musculus

&lt;400&gt; 89

Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Thr Ser Gly  
 1 5 10 15

Glu Xaa Xaa Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Xaa Pro  
 20 25 30

Xaa Ser Thr Arg Asp Gly Xaa Leu Leu Ile Ala Gly Ser Glu Ala Pro  
 35 40 45

Lys Arg Gly Xaa Xaa Leu Ser Lys Pro Arg Thr Gly Gly Ala Gly Xaa  
 50 55 60  
 Gly Lys Pro Xaa Xaa Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
 65 70 75 80  
 Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Xaa Ala Phe Ile Tyr His  
 85 90 95  
 Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
 100 105 110  
 Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile  
 115 120 125  
 Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
 130 135 140  
 Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
 145 150 155 160  
 Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu  
 165 170 175  
 Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe  
 180 185 190  
 Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met  
 195 200 205  
 Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val  
 210 215 220  
 Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe  
 225 230 235 240  
 Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly  
 245 250 255  
 Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu  
 260 265 270  
 Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp  
 275 280 285  
 Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe  
 290 295 300  
 Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val  
 305 310 315 320  
 Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala  
 325 330 335

43

Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser  
 340 345 350  
 Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Xaa Thr Val Thr  
 355 360 365  
 Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu  
 370 375 380  
 Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys  
 385 390 395 400  
 Ser Gly Leu Thr Phe Arg Lys Glu Pro Gln Pro Glu Pro Ser Pro Ser  
 405 410 415  
 Pro Arg Gly Met Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr  
 420 425 430  
 Val Arg Arg Ser Pro Ser Ala Asp Gln Ser Leu Asp Asp Ser Pro Ser  
 435 440 445  
 Lys Val Pro Lys Ser Trp Ser Phe Gly Asp Arg Ser Arg Thr Arg Gln  
 450 455 460  
 Ala Phe Arg Ile Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala  
 465 470 475 480  
 Ser Leu Pro Gly Glu Asp Ile Val Glu Asp Asn Lys Ser Cys Asn Cys  
 485 490 495  
 Glu Phe Val Thr Glu Asp Leu Thr Pro Gly Leu Lys Xaa Ser Ile Arg  
 500 505 510  
 Ala Val Cys Xaa Met Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu  
 515 520 525  
 Ser Leu Arg Pro Tyr Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala  
 530 535 540  
 Gly His Leu Asp Met Leu Ser Arg Ile Lys Ser Leu Gln Thr Arg Val  
 545 550 555 560  
 Asp Gln Ile Val Gly Arg Gly Pro Thr Ile Thr Asp Lys Asp Arg Thr  
 565 570 575  
 Lys Gly Pro Ala Glu Thr Glu Leu Pro Glu Asp Pro Ser Met Met Gly  
 580 585 590  
 Arg Leu Gly Lys Val Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu  
 595 600 605  
 Asp Phe Leu Val Ser Ile Tyr Thr Gln Arg Met Gly Ile Gln Pro Ala  
 610 615 620

44

Glu Thr Glu Ala Tyr Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro  
 625 630 635 640  
 Tyr His Ser Pro Glu Asp Ser Arg Asp His Ala Asp Lys His Gly Cys  
 645 650 655  
 Ile Ile Lys Ile Val Arg Ser Thr Ser Ser Thr Gly Gln Arg Asn Tyr  
 660 665 670  
 Ala Ala Pro Pro Ala Ile Pro Pro Ala Gln Cys Pro Pro Ser Thr Ser  
 675 680 685  
 Trp Arg Gln Ser His Gln Arg His Gly Thr Ser Pro Val Gly Asp His  
 690 695 700  
 Gly Ser Leu Val Arg Ile Pro Pro Leu Pro Ala His Glu Arg Ser Leu  
 705 710 715 720  
 Ser Ala Tyr Gly Gly Gly Asn Arg Ala Ser Thr Glu Phe Leu Arg Leu  
 725 730 735  
 Glu Gly Thr Pro Ala Cys Arg Pro Ser Glu Ala Ala Ala Gly Gln Arg  
 740 745 750  
 His Val His Leu His  
 755

&lt;210&gt; 90

&lt;211&gt; 2814

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (202)..(2811)

&lt;400&gt; 90

ctccccaccc gacctcctct ggcggggggg agggccgcct ttgcctgctt ttgggggggt 60

gggcgggggag gggcgcgcg atcatggcat tggagttccc gggcttgcag ccgccgccgc 120

cgccctcgctcc acgtacccca agtgctccta cttcccggag cagcagcgga gaaggcgaag 180

cgccccggtgg gggcgaggca g atg ggt ctc aag gct cgc agg gcg gct ggc 231  
 Met Gly Leu Lys Ala Arg Arg Ala Ala Gly  
 1 5 10

ggg ggc ggc ggc gaa ggg ggc ggc gga ggc ggc ggg gcc gct aac cct 279  
 Gly Gly Gly Gly Glu Gly Gly Gly Gly Gly Gly Ala Ala Asn Pro  
 15 20 25

gct gga ggg gac tcg gcg gtg gcc ggc gac gag gag cgg aaa gtg ggg 327  
 Ala Gly Gly Asp Ser Ala Val Ala Gly Asp Glu Glu Arg Lys Val Gly  
 30 35 40

45

ctg gcg cca gga gac gtg gag caa gtc acc ttg gcg cta ggg gcc gga	375
Leu Ala Pro Gly Asp Val Glu Gln Val Thr Leu Ala Leu Gly Ala Gly	
45 50 55	
gcc gac aaa gac ggg acc ctg ctg ctg gag ggc ggt ggc cgc gaa gag	423
Ala Asp Lys Asp Gly Thr Leu Leu Leu Glu Gly Gly Gly Arg Glu Glu	
60 65 70	
ggg cag agg agg acc ccg cag ggc atc ggg ctc ctg gca aag acc ccc	471
Gly Gln Arg Arg Thr Pro Gln Gly Ile Gly Leu Leu Ala Lys Thr Pro	
75 80 85 90	
ctg agc cgc cca gtc aag agg aac aac gcc aag tac agg cgc atc caa	519
Leu Ser Arg Pro Val Lys Arg Asn Asn Ala Lys Tyr Arg Arg Ile Gln	
95 100 105	
act ttg atc tat gac gcc ctg gag aga ccg cgg ggc tgg gcg ctg ctc	567
Thr Leu Ile Tyr Asp Ala Leu Glu Arg Pro Arg Gly Trp Ala Leu Leu	
110 115 120	
tac cac gcg ctt gtg ttc ctg att gtc ctg gga tgc ttg att ctg gcc	615
Tyr His Ala Leu Val Phe Leu Ile Val Leu Gly Cys Leu Ile Leu Ala	
125 130 135	
gtg ctc acc acc ttc aag gaa tat gag act gtg tct gga gac tgg ctt	663
Val Leu Thr Thr Phe Lys Glu Tyr Glu Thr Val Ser Gly Asp Trp Leu	
140 145 150	
ttg ctg ctg gaa aca ttt gct att ttc atc ttt gga gct gag ttt gct	711
Leu Leu Leu Glu Thr Phe Ala Ile Phe Ile Phe Gly Ala Glu Phe Ala	
155 160 165 170	
ttg agg atc tgg gct gca gga tgt tgc tgt cga tac aaa ggc tgg cgt	759
Leu Arg Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Lys Gly Trp Arg	
175 180 185	
gga cgg cta aag ttt gcc agg aag ccc ctg tgc atg ttg gac atc ttc	807
Gly Arg Leu Lys Phe Ala Arg Lys Pro Leu Cys Met Leu Asp Ile Phe	
190 195 200	
gta ctg att gcc tct gtg cca gtg gtt gcc gtg gga aac cag ggc aat	855
Val Leu Ile Ala Ser Val Pro Val Val Ala Val Gly Asn Gln Gly Asn	
205 210 215	
gtc ttg gcc acc tcc ctg cga agc ctt cgc ttc ctg cag atc ctg cgc	903
Val Leu Ala Thr Ser Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg	
220 225 230	
atg ctt cga atg gat agg agg ggt ggc acc tgg aag ctc ctg ggc tcg	951
Met Leu Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser	
235 240 245 250	

46

gct atc tgt gcc cac agc aaa gaa ctc atc act gcc tgg tac ata ggc	999
Ala Ile Cys Ala His Ser Lys Glu Leu Ile Thr Ala Trp Tyr Ile Gly	
255 260 265	
ttc ctg aca ctc atc ctt tct tca ttt ctt gtc tac ctg gtg gag aag	1047
Phe Leu Thr Leu Ile Leu Ser Ser Phe Leu Val Tyr Leu Val Glu Lys	
270 275 280	
gat gtg cca gaa atg gat gcc caa gga gag gag atg aag gag gag ttt	1095
Asp Val Pro Glu Met Asp Ala Gln Gly Glu Glu Met Lys Glu Glu Phe	
285 290 295	
gag acc tat gca gat gct ctg tgg tgg ggc ctg atc aca ctg gcc acc	1143
Glu Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu Ile Thr Leu Ala Thr	
300 305 310	
att ggt tat gga gac rag aca cct aaa acc tgg gaa gga cgt ctg att	1191
Ile Gly Tyr Gly Asp Xaa Thr Pro Lys Thr Trp Glu Gly Arg Leu Ile	
315 320 325 330	
gct gcc acc ttt tct tta atc ggc gtc tcc ttt ttt gcc ctt ccg gca	1239
Ala Ala Thr Phe Ser Leu Ile Gly Val Ser Phe Phe Ala Leu Pro Ala	
335 340 345	
ggc atc ctt ggc tca gga ctg gca ctg aag gtt cag gag cag cac cgt	1287
Gly Ile Leu Gly Ser Gly Leu Ala Leu Lys Val Gln Glu Gln His Arg	
350 355 360	
cag aag cac ttt gag aag aga agg aag cca gct gcg gaa ctc atc cag	1335
Gln Lys His Phe Glu Lys Arg Arg Lys Pro Ala Ala Glu Leu Ile Gln	
365 370 375	
gct gcc tgg aga tat tat gct acc aac ccc aac agg ttg gat ctg gtg	1383
Ala Ala Trp Arg Tyr Tyr Ala Thr Asn Pro Asn Arg Leu Asp Leu Val	
380 385 390	
gca acc tgg aga tct tat gaa tca gtt gtc tct ttc cca ttc ttc agg	1431
Ala Thr Trp Arg Ser Tyr Glu Ser Val Val Ser Phe Pro Phe Phe Arg	
395 400 405 410	
aaa gaa caa ctg gaa gca gca gcc agc caa aag ctg ggt ctc ttg gat	1479
Lys Glu Gln Leu Glu Ala Ala Ala Ser Gln Lys Leu Gly Leu Leu Asp	
415 420 425	
cgg gtt cgc ctt tct aat cct cgt ggt agc aat act aaa gga aag cta	1527
Arg Val Arg Leu Ser Asn Pro Arg Gly Ser Asn Thr Lys Gly Lys Leu	
430 435 440	
ttt acc cct ctg aat gta gat gcc ata gaa gaa agc cct tcc aaa gag	1575
Phe Thr Pro Leu Asn Val Asp Ala Ile Glu Glu Ser Pro Ser Lys Glu	
445 450 455	
cca aag cct gtt ggc tta aac aat aaa gag cgt ttc cgc acc gcc ttc	1623
Pro Lys Pro Val Gly Leu Asn Asn Lys Glu Arg Phe Arg Thr Ala Phe	
460 465 470	

cgc atg aaa gcc tac gct ttc tgg cag agw tct gaa gat gct ggg aca	1671
Arg Met Lys Ala Tyr Ala Phe Trp Gln Xaa Ser Glu Asp Ala Gly Thr	
475 480 485 490	
ggc gac ccc atg gca gaa gac agg ggc tat ggg aat gac wtc ctc att	1719
Gly Asp Pro Met Ala Glu Asp Arg Gly Tyr Gly Asn Asp Xaa Leu Ile	
495 500 505	
gma gac atg atc cct rcc ctw aag gct gcc atc cga gct gtc aga att	1767
Xaa Asp Met Ile Pro Xaa Xaa Lys Ala Ala Ile Arg Ala Val Arg Ile	
510 515 520	
cta cag ttc cgt cta tat aaa aaa aag ttc aag gag acg ttg agg cct	1815
Leu Gln Phe Arg Leu Tyr Lys Lys Lys Phe Lys Glu Thr Leu Arg Pro	
525 530 535	
tat gat gtg aaa gat gtg att gag cag tat tcg gcc gga cat ctt gac	1863
Tyr Asp Val Lys Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu Asp	
540 545 550	
atg ctt tcc agg ata aag tac cta cag aca aga ata gat atg att ttc	1911
Met Leu Ser Arg Ile Lys Tyr Leu Gln Thr Arg Ile Asp Met Ile Phe	
555 560 565 570	
acc cct gga cct cca tcc act cca aaa cat aag aag tct cag aaa ggg	1959
Thr Pro Gly Pro Pro Ser Thr Pro Lys His Lys Lys Ser Gln Lys Gly	
575 580 585	
aca gca ttt acc tac cca tcc cag cag tct cca agg aat gaa cca tat	2007
Thr Ala Phe Thr Tyr Pro Ser Gln Gln Ser Pro Arg Asn Glu Pro Tyr	
590 595 600	
gta gcc agg gca gcc aca tca gaa act gaa gac caa agc atg atg ggg	2055
Val Ala Arg Ala Ala Thr Ser Glu Thr Glu Asp Gln Ser Met Met Gly	
605 610 615	
aag ttt gta aaa gtt gaa aga cag gtt cat gac atg ggg aag aaa ctg	2103
Lys Phe Val Lys Val Glu Arg Gln Val His Asp Met Gly Lys Lys Leu	
620 625 630	
gac tyc ctc gtg gac atg cat atg cag cat atg gaa cgc tta cag gta	2151
Asp Xaa Leu Val Asp Met His Met Gln His Met Glu Arg Leu Gln Val	
635 640 645 650	
cat gtc act gag tac tac cca act aar ggg gcc tcc tcc cca gcc gaa	2199
His Val Thr Glu Tyr Tyr Pro Thr Xaa Gly Ala Ser Ser Pro Ala Glu	
655 660 665	
ggg gag aag aaa gaa gac aac agg tac tct gat ttg aaa acc atc atc	2247
Gly Glu Lys Lys Glu Asp Asn Arg Tyr Ser Asp Leu Lys Thr Ile Ile	
670 675 680	

tgc aac tac tca gag aca ggg ccc cct gac cct cct tac agc ttc cac	2295
Cys Asn Tyr Ser Glu Thr Gly Pro Pro Asp Pro Pro Tyr Ser Phe His	
685 690 695	
cag gtg ccc atc gac aga gtt ggc cct tac ggg ttt ttt gca cat gat	2343
Gln Val Pro Ile Asp Arg Val Gly Pro Tyr Gly Phe Phe Ala His Asp	
700 705 710	
cct gtg aaa ctg acc cga ggg gga ccc agt tct aca aag gct caa gct	2391
Pro Val Lys Leu Thr Arg Gly Gly Pro Ser Ser Thr Lys Ala Gln Ala	
715 720 725 730	
aac ctt ccc tcc tcg gga agt aca tat gca gag agg ccc aca gtc ctg	2439
Asn Leu Pro Ser Ser Gly Ser Thr Tyr Ala Glu Arg Pro Thr Val Leu	
735 740 745	
ccc atc ttg act ctt ctg gac tca tgt gtg agc tac cac tcc cag aca	2487
Pro Ile Leu Thr Leu Leu Asp Ser Cys Val Ser Tyr His Ser Gln Thr	
750 755 760	
gaa ctg caa ggt ccc tat tcg gac cac atc tcg ccc cgc cag aga cgc	2535
Glu Leu Gln Gly Pro Tyr Ser Asp His Ile Ser Pro Arg Gln Arg Arg	
765 770 775	
agc atc act agg gac agt gat aca cct ctg tcc ctc atg tcc gtc aat	2583
Ser Ile Thr Arg Asp Ser Asp Thr Pro Leu Ser Leu Met Ser Val Asn	
780 785 790	
cac gag gaa ctg gag cgg tct cca agt ggc ttc agc atc tca caa gac	2631
His Glu Glu Leu Glu Arg Ser Pro Ser Gly Phe Ser Ile Ser Gln Asp	
795 800 805 810	
aga gat gat tat gta ttt ggc ccc agt ggg gga tcg agc tgg atg ggg	2679
Arg Asp Asp Tyr Val Phe Gly Pro Ser Gly Gly Ser Ser Trp Met Gly	
815 820 825	
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Phe Thr Pro Ser Gly Ser Met Pro Met Ser Ser Thr Gly Asp Gly Ile	
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51

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Ile Glu Gln Tyr Ser Ala Gly His Leu Asp Met Leu Ser Arg Ile Lys
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His Met Gln His Met Glu Arg Leu Gln Val His Val Thr Glu Tyr Tyr
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Pro Thr Xaa Gly Ala Ser Ser Pro Ala Glu Gly Glu Lys Lys Glu Asp
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Asn Arg Tyr Ser Asp Leu Lys Thr Ile Ile Cys Asn Tyr Ser Glu Thr
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Leu Arg Pro Tyr Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala Gly	
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 Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
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&lt;210&gt; 106

&lt;211&gt; 232

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 106

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&lt;210&gt; 107

&lt;211&gt; 128

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 107

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&lt;210&gt; 108

&lt;211&gt; 318

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 108

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68

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&lt;210&gt; 119

&lt;211&gt; 973

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 119

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&lt;210&gt; 120

&lt;211&gt; 1117

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 120

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&lt;211&gt; 1083

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 121

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&lt;210&gt; 122

&lt;211&gt; 819

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 122

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<211> 1232

<212> DNA

<213> Homo sapiens

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&lt;210&gt; 125

&lt;211&gt; 1172

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 125

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&lt;213&gt; Homo sapiens

&lt;400&gt; 129

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/22375

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1, 325; 530/387.1; 536/23.1, 24.3, 24.31; 800/3, 13, 14, 18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	STOFFEL et al. Epilepsy genes: excitement traced to potassium channels. Nature Genetics. January 1998, Vol. 18, No. 1, pages 6-8, entire document.	1-70
Y, P	SINGH et al. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nature Genetics. January 1998, Vol. 18, No. 1, pages 25-29, entire document.	1-70
Y, P	CHARLIER et al. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nature Genetics. January 1998, Vol. 18, No. 1, pages 53-55, entire document.	1-70



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

06 DECEMBER 1998

Date of mailing of the international search report

22 JAN 1999

Name and mailing address of the ISA/US  
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/22375

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEWIS et al. Localization of a gene for a glutamate binding subunit of a NMDA receptor (GRINA) to 8q24. Genomics. 1996, Vol. 32, No. 1, pages 131-133, entire document.	1-70
Y	STEINLEIN et al. Exon-intron structure of the human neuronal nicotinic acetylcholine receptor alpha4 subunit (CHRNA4). Genomics. 1996, Vol. 32, No. 1, pages 289-294, entire document.	1-70
Y	STEINLEIN.O. Detection of a CfoI polymorphism within exon 5 of the human neuronal nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4). Hum. Genet. 1995, Vol. 96, page 130, entire document.	1-70
Y	STEINLEIN et al. Benign familial neonatal convulsions: confirmation of genetic heterogeneity and further evidence for a second locus on chromosome 8q. Hum. Genet. 1995, Vol. 95, pages 411-415, entire document.	1-70

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/22375

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 1, 2, 4-14, 16-19, 21-24, 26, 28, 32, 33, 35-37, 40, 42, 44, 48, 51, 53, 55, 57, 59, 63, 65-70  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.  
No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/22375

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 21/04; C07K 16/00; C12N 15/00, 15/11, 15/63, 15/85, 15/86

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/320.1, 325; 530/387.1; 536/23.1, 24.3, 24.31; 800/3, 13, 14, 18

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS

STN (file: medicine)

search terms: juvenile myoclonic epilepsy, JME, rolandic epilepsy, benign familial neonatal convulsions, BFNC, KCNQ2, KCNQ3, KVEBN1, KVEBN2

BOX 1. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

Insofar as the claims recite SEQ ID NO.'s or depend from claims that recite SEQ ID NO.'s, the claims were found to be unsearchable, because the computer readable format (CRF) was found to be technically bad. Thus, the claims were searched based on the description provided in the disclosure and keywords from the claims to the best of the ability of the examiner.